IMPROVEMENT AND REFINEMENT OF EXISTING CHOLESTEROL TOLERANCE TEST

THESIS

FOR

DOCTOR OF MEDICINE

(MEDICINE)

BUNDELKHAND UNIVERSITY
JHANSI (U. P.)

1990 NIRBHAI KUMAR

C E R T I F I C A T E

This is to certify that the work entitled "IMPROVEMENT AND REFINEMENT OF EXISTING CHOLESTEROL TOLERANCE TEST" which is being submitted as a thesis for M.D.(Medicine) examination, 1990 of Bundelkhand University by Dr. Nirbhai Kumar, has been carried out in the department of Medicine, M.L.B. Medical College, Jhansi.

He has put in the necessary stay in the department as per University regulations.

Dated: 15.9., 1989.

(R. C. Arora)
M.D., D.Sc.,
Professor and Head,
Department of Medicine,
M.L.B. Medical College,
Jhansi.

CERTIFICATE

This is to certify that the work entitled "IMPROVEMENT AND REFINEMENT OF EXISTING CHOLESTEROL TOLERANCE TEST", which is being submitted as a thesis for M.D. (Medicine) examination, 1990 of Bundelkhand University by Dr. Nirbhai Kumar, has been carried out under my direct supervision and guidance. The techniques and statistical methods used were undertaken by the candidate himself and were checked by me from time to time.

Dated: 15.9 , 1989.

(R. C. Arora)
M.D., D.Sc.,
Professor and Head,
Department of Medicine,
M.L.B. Medical College,
Jhansi.

(GUIDE)

CERTIFICATE

This is to certify that the work entitled "IMPROVEMENT AND REFINEMENT OF EXISTING CHOLESTEROL TOLERANCE TEST" has been carried out by Dr. Nirbhai Kumar under my direct supervision and guidance. The techniques and statistical methods used in this thesis have been undertaken by the candidate himself and checked by me from time to time.

Dated: 15.9.,1989.

(Navnit Agarwal)
M.D.,
Lecturer in Medicine,
M.L.B. Medical College,
Jhansi.

(CO-GUIDE)

CERTIFICATE

This is to certify that the work entitled "IMPROVEMENT AND REFINEMENT OF EXISTING CHOLESTEROL TOLERANCE TEST" has been carried out by Dr. Nirbhai Kumar under my direct supervision and guidance. The techniques and statistical methods used in this thesis have been undertaken by the candidate himself and checked by me from time to time.

Dated: 15.9.,1989.

(Navnit Agarwal)
M.D.,
Lecturer in Medicine,
M.L.B. Medical College,
Jhansi.

(CO-GUIDE)

C E R T I F I C A T E

This is to certify that the work entitled "IMPROVEMENT AND REFINEMENT OF EXISTING CHOLESTEROL TOLERANCE TEST" has been carried out by Dr. Nirbhai Kumar under my direct supervision and guidance. The techniques and statistical methods used in this thesis have been undertaken by the candidate himself and checked by me from time to time.

Dated: 15.9. ,1989.

Sumita Arora

(Sumita Arora)
M.S.,
Reader,
Department of Obstetrics and Gynaecology,
M.L.B. Medical College,
Jhansi.

(CO-GUIDE)

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

The most pleasant duty in a work like this is to remember and ruminate my obligations to all those who have made its completion possible.

I consider it a previlege to express with a deep sense of gratitude my indebtedness to my Chief Guide Prof. R.C. Arora, MD., MCCP, FICA, D.Sc., Head, Department of Medicine, M.L.B. Medical College, Jhansi for the untiring help, able guidance, astute and unfailing scruitiny and careful supervision provided by him during the entire period of this study. It would be no exaggeration to state that but for his endless patience, invaluable suggestions and his dedication to provide me with every opportunity to learn the subject, this endeavour would never have seen light. Things that I have learnt from him will take me a long way in my carrier. He has not only been a valuable and immense help in my thesis but a memory that would be everlasting.

Words fail to express my deepest sense of gratitude and sincere most thanks to my respected Coguide Dr. Navnit Agarwal, M.D., Lecturer in Medicine, M.L.B. Medical College, Jhansi, who ungrudgingly rendered his valued criticism and learned advice as and when needed till completion of this work. His

efforts to get me over my initial hesitations and inspire in to me a sense of confidence were overwhelming. His cheerful attitude and reassuring presence in helping me tide over my difficulty faced in the work shall remain in my thoughts for ever.

My sincere most thanks are due to Dr.(Mrs.) Sunita Arora, M.S., Reader in Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi who always helped me in every possible way to achieve my target. Her valuable suggestions, constructive criticism and meticulous attention have gone a long way towards the success of this work.

I find myself indebted to Prof. D.N. Mishra, MD, MNAMS, FCCP, Professor in Medicine., Dr. G. D. Shukla, MD, MNAMS, Ph.D., Lecturer in Psychiatry, Dr. P. K. Jain, MD, MNAMS, Lecturer in Medicine, Dr. Praveen Kumar, MD, Dip. Card., DM(Cardl), Lecturer in Cardiology, Dr. T.V.S. Arya, MD, Lecturer in Medicine, Dr. R. K. Garg, M.D., Pool Officer in Medicine, for providing a constant source of inspiration and enthusiasm to complete the present work.

I will be failing in my duty if I do not extend my sincere most thank to Dr. Sanjay Lakhtakia, M.B.B.S., Resident, Department of Medicine for his

invaluable, meticulous and consistent help throughout the period of this study. In no less degree I thank to my junior colleagues specially Bipin Goel, R.K.Verma, Vivek Agarwal, Manu Tandon and Vimal Agha who have spared no efforts in making this project a success.

It gives me special pleasure to acknowledge the invaluable help and constant inspiration provided by my parents. I also owe my thanks to my wife who provided moral support and encouragment during my hours of desperation due to overwhelming problems and time consuming process.

I am also thankful to Mr. B.M. Sharma for his las assistance and Mr. Phool Chandra Sachan for bringing out such a neatly typed script.

Lastly, I thank to all the subjects who volunteered in this project.

Dated : 14.9.89

Nirbhai Kumar

(Nirbhai Kumar)

C O N T E N T S

INTRODUCTION

INTRODUCTION

Within physiological limits, the levels of basal serum cholesterol do not help in predicting an individual risk of developing atherosclerosis related complications like coronary artery disease(CAD). Over more than forty per cent of young patients of documented CAD do not reveal raised fasting cholesterol level (Gregory et al, 1983), yet they have rampant atherogenous vascular involvement. This indicates that importance of basal fasting cholesterol level in assessing risk for CAD has perhaps been over emphasised.

Zilversmit (1973) postulated that atherogenesis may be a postprandial phenomenon. Transient postprandial rise of beta VLDL, chylomicron and formation of several species of unusual lipoproteins, may cause repeated cholesterol deposition in cells in arterial wall over the years, while fasting cholesterol value may remain well within normal range over the same duration.

These facts clearly indicate that it is more important to study postprandial response of serum cholesterol and not merely the fasting levels. Considering these facts previous workers in our department (Arora and Mangal et al, 1988) tried to evolve a simple cholesterol tolerance test by single point feeding of high cholesterol fat diet (HCFD) and then observing

behaviour of changes in lipid lipoprotein profiles at first and third postprandial hour.

This test though simple has many flaws and limitations. Some of these are as follows :

1. Single dose HCFD given in the test consisted of 3 eggs plus 200 ml of milk (about 775 mg cholesterol and 30 gm fat). For formulation of a test it is important to use minimum amount of cholesterol fat diet stress that would produce significant changes (though magnitudes of such changes may be less). More so such large cholesterol load does not seem practical not acceptable to every one and probably useless.

2. Large segment of ever population is vegetarian so evolving a test based on egg diet is not justified. Cholesterol fat diet in some other form like crystalline cholesterol butter and milk or a combined formula diet should be used, which will be acceptable to all members of the community.

3. The proposed cholesterol fat tolerance test(CFT), takes into account only two postprandial samples - one at one hour and another at three hours. We do not know presently that what is the time gap after a HCFD, at which peak level is achieved and after what time these changes disappear. Thus choosing these two postprandial samples seems arbitrary, erratic and probably without a proper rationale.

4. Unlike glucose tolerance test, the response of serum cholesterol after feeding HCFD is not consistent, uniform and reproducible. The latter factor is of vital importance and if a test in an individual has not been shown to be reproducible, the validity of its importance is questionable.
5. There has been a diverse behaviour of changes in lipid profile after feeding HCFD in the said test. In about half of the cases there become a fall of serum total cholesterol (STC) and low density lipoprotein (LDL), after feeding HCFD but the remaining half show either a rise or no change. What should be considered a normal behaviour after feeding, remains unanswered.
6. Apart from fat and cholesterol, other dietary constitutes protein and carbohydrate induced lipid lipoprotein changes should also be observed so as to make a comprehensive comparison between these changes.

The aim of this study is to correct different flaws and limitations of proposed single dose CFT by different clinical and pilot studies.

OBJECTS OF THE STUDY

1. To find out whether plasma lipoprotein changes induced by egg cholesterol can be duplicated by crystalline cholesterol and food substances other than egg cholesterol.

2. To determine the quantitative and qualitative spectrum of the change in plasma lipid profile which would include earliest change, peak change and plateau of plasma lipoprotein profile by studying serum lipid profiles in postprandial samples taken at different intervals.

3. To assess whether the results of single dose cholesterol tolerance test are reproducible and predictable.

4. To make comparison of single point cholesterol feeding with prolonged feeding (7 15 days) in same individuals.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Hyperlipoproteinemia is considered to be one of the most important factor in development of atherosclerotic disorders. Numerous studies have implicated altered levels of plasma lipoproteins in pathogenesis of atherosclerosis. In particular, low level of high density lipoprotein, high level of low density lipoprotein and high level of serum total cholesterol appear to be high risk factor.

Atherosclerosis is a degenerative process associated with advancing years, mainly affecting larger arteries, particularly the coronary and cerebrals. The lesion of atherosclerosis passes through many phases - fatty streak, fibrous plaque and finally advanced lesion.

The changes in plasma lipoproteins after short term and long term feeding of cholesterol fat rich diet have been extensively studied in the past. Different types of experimental diets - crystalline cholesterol, egg cholesterol, butter, milk - cholesterol formula diets based on oil and protein and carbohydrate diets have been used to assess individuals response in plasma lipid profile (Connor et al, 1961; Deborah Applebaum-Bowden, 1979; Beveridge, 1971).

EFFECT OF FEEDING ON SERUM TOTAL CHOLESTEROL(STC)

Effect of long term and short term feeding of

diet rich in cholesterol has been extensively studied over the past 30 years. Dietary fat and cholesterol causes changes in specific lipoprotein in a variety of animal species (Mahley et al, 1977), quantitatively, a change in specific lipoprotein may be dramatic in one species than in another.

Ancelkeys and Antersen et al (1956) concluded that serum cholesterol level is essentially independent of cholesterol intake over the whole range of natural human diets. But later on it was proved beyond doubt that feeding cholesterol rich diet for 2-8 weeks raises total serum cholesterol in blood (Arora et al, 1986; Messinger et al, 1950; Conner et al, 1961; Deborah Applebaum et al, 1979).

In an earlier report, Bruhn (1940) observed a 20% rise in mean cholesterol level after a fat load. Effect of high cholesterol fat load on postprandial cholesterol levels has also been studied in the past by several workers, but insignificant difference has been found between postprandial and 10-14 hours fasting value (Albrink and Man, 1956; Pomeranze et al, 1954 and Schilling et al, 1964).

Textured vegetable proteins lowered total serum cholesterol in hyper cholesterolemic subjects with no change or slight elevation of HDL. Little effects were observed in normolipidemic subjects (Sirtori et al,

1985).

The replacement of animal protein with vegetable protein in the diet has been suggested to reduce the diet linked atherogenic (Carroll, 1982). However, sacks et al, (1983) found no appreciable correlation between total intake of protein, when consumed above minimum requirement and serum cholesterol level.

In one study, isocaloric replacement of starch with sucrose in mixed diet did not lead to changes in serum cholesterol (Mann and Truswell, 1972).

EFFECT OF FEEDING ON HIGH DENSITY LIPOPROTEIN(HDL)

Borden et al (1964) reported enhanced levels of HDL in rats fed cholesterol while Haff et al (1962) and Kritchersy (1965) reported no change in HDL levels in cholesterol fed rats.

There is evidence that substitution of large quantities of poly-unsaturated fat for saturated fat in diet can result in lower levels of HDL lipids and proteins (Nichaman et al, 1967). An increase in the P/S ratio from 0.25:1 to 4:1 in food diet fed to four normal subjects for five weeks resulted in reduction of HDL and apolipoproteins A-I concentration of 33 and 21% respectively, with an associated reduction in HDL_2:HDL_3 ratio (Shephered et al, 1978). Other studies have however reported either no change(lewis, 1978; Shore et al, 1981) or increase (Jackson and Glueck, 1980) in

levels of HDL with feeding of diets enriched in poly-unsaturated fat.

High dietary intake of cholesterol, in the form of 3-6 egg yolk per day, has been reported to produce increase in apolipoprotein E-containing HDL sub species in human (Mahley et al, 1978). Tan et al (1974) showed that level of HDL and serum apolipoprotein A-I, but not apolipoprotein E increased with the feeding of diets high in both cholesterol and saturated fat.

Recently it has been shown that HDL apolipo-protein A-I levels increased when fat was consumed in divided doses over a period of 10 hours, but not when the same amount of fat was ingested as a single load (Kay et al, 1980).

HDL AS A PREDICTOR OF CAD

The ability of HDL to predict the development of coronary atherosclerosis has been estimated to be four times greater than total cholesterol. Each 10 mg/dl change in cholesterol concentration results in 50 percent alteration in cardiovascular risk (Yaari S, Goldbourt U, Even-Zohar et al, 1981). The ratio of total cholesterol to HDL cholesterol also is about as efficient as any other lipid profile in predicting the future development of CAD(Gordon T, Kannel WB, Castelli WP et al, 1981).

LOW DENSITY LIPOPROTEIN(LDL) CHANGES ON FEEDING

Diet high in fat and cholesterol cause an elevation in LDL in most animals (Mahley, 1978). The response in man varies, but in those subjects who have an elevation in plasma cholesterol, there is an elevation in plasma LDL levels. Deborah Applebaum et al (1979) demonstrated significant rise of LDL level in human volunteers after feeding 5000 mg of egg yolk cholesterol per day for 30 days.

Age related difference in rise of LDL was demonstrated by Arora and Gupta et al (1987). They found that rise of total serum cholesterol after feeding HCFD for one week was much more pronounced in young (20-30 years) volunteers with major portion of rise being contributed by increased HDL. Contrary, in older age person the rise of STC was less marked with LDL contribution, mainly in the increased levels.

Band et al (1981) demonstrated that there was significant fall in level of LDL in five volunteers 3 hours and 5 hours after taking butter diet. They attributed this fall due to defect in VLDL hydrolysis by serum lipases and due to metabolic blocking in liver or adipose tissues.

In addition to this, this has also been shown that diet induced LDL molecules have large molecular size than those on low fat cholesterol diet (Rudel et al. 1979). Clair and Leight (1978) have reported that the

diet induced, large LDL are capable of stimulating cholesteryl esterification and accumulation in smooth muscle cells to a greater extent than are normal LDL. Diet induced apoprotein fraction changes in LDL have also been reported(Mahley et al, 1977; Rudel et al,1979).

CONCEPT OF LDL RECEPTOR IN CONTROL OF SYC

It is now considered that LDL receptors play a pivotal role in regulating the level of serum cholesterol (Kita et al, 1982). In rabbits, rats and hamsters more than half of the total LDL receptors are located in the liver. However, the precise distribution of these receptors in man is unknown.

Hepatic LDL receptors are suppressed whenever the liver's content of cholesterol increases or its demand for cholesterol is reduces. Thus receptor suppression occurs when a high cholesterol diet is consumed (Mui et al, 1981) or when bile acids are infused (Angelin et al, 1983). Conversely LDL receptors increases when hepatic cholesterol synthesis is blocked by drugs compactin or (Goldstein et al, 1982 and Bilheimer, et al, 1983), when bile acid binding resins are given (Shepherd et al, 1980). Fasting has also been shown to suppress LDL receptor in rabbits (Goldstein, 1982). LDL receptors can be stimulated by thyroxine(Thompson, 1981) and by pharmacologic doses of estrogen(Winder,1980).

All the changes in receptor activity alter the rate of uptake of LDL by the liver and cause reciprocal changes in plasma LDL levels. Whenever hepatic LDL receptors are suppressed, the plasma LDL level rises, conversely, whenever, these receptors are induced, the plasma LDL levels fall. In familial hypercholesterolemia the basic defect is reduced number of LDL receptors. In normal person about 45% of the plasma LDL pool is removed from the plasma daily by the receptors, whereas in familial hypercholesterolemia heterozygotes it is about 15%. This receptor deficiency results in accumulation of LDL into the plasma, leading to raised level and premature atherosclerosis.

FEEDING INDUCED CHANGES IN SERUM TRIGLYCERIDES(STG) AND VERY LOW DENSITY LIPOPROTEIN (VLDL)

Rise in the triglyceride level after fat ingestion has been reported after giving different amounts of the fat load and measuring the blood levels at different time interval (Mikkila and Kanttinen,1962; Denborrough, 1963).

Cleofsky et al (1976) noted a biphasic plasma triglyceride curve with an initial peak occurring 1 to 3 hours after feeding and a secondary peak after 4 to 7 hours. The primary peak was accounted by increase in chylomicron level in more than 98% cases, whereas secondary peak represented rise in very low density lipoprotein (VLDL)level in 82%.

Hamokrauss et al (1957) did not reveal any significant changes in serum total cholesterol after a heavy fat cholesterol load, but found significant difference in triglyceride levels.

Arora and Kusherba et al (1957) put forward the concept of triglyceride tolerance test which showed significant difference in peak levels of STG in normal healthy patient of IHD and that of diabetes.

Diet prior to the loading test meal, may be decisive under metabolic ward conditions, significant difference in fat tolerance has been reported in healthy subjects on an isocaloric diet, when the daily fat intake per kg of body weight was varied from 0.1-2 g(Havel,1957).

Test meal composition has also been shown to affect serum triglyceride level significantly. In human beings, glucose one hour and half an hour before as well as one and a half hour after a fat meal reduced or even eliminated the serum triglyceride rise (Albrink and Man, 1956). Glucose addition to 131 I - labelled triolein caused a flatter triglyceride curve as compared to ingestion of the latter only (Berkowitz et al, 1959).

Long term studies on the effect of dietary protein on lipid level indicate that low protein intake is accompanied by a depression of serum lipids (Olsen et al, 1957).

George C Lin et al (1983) incorporated 2 levels of dietary carbohydrate (40% and 60%) in the usual diet for 15 days in 8 patients suffering with endogenous hyper triglyceridemia. Fasting blood samples were drawn on days 13, 14 and 15 of each dietary period. In addition, samples were also drawn 3 hours before and after noon meal on days 14 and 15. They reported that low fat, high carbohydrate diet accentuates the metabolic risk for CAD that is already present in patients of endogenous hyper-triglyceridemia. They also reported that rise in plasma triglyceride is mainly depends upon total calorie intake.

Arora and Kushwaha (1967) proposed a 'triglyceride tolerance test'. The workers gave a fat load and found a significant peak at 5 hour in healthy volunteers.

CHOLESTEROL FAT TOLERANCE TEST

The concept of such test is now new. Newman (1907) studied the quantitative lipid changes in form of chylomicron count after giving a fat load. Krakowitz (1963) pointed out that radioactive fat tolerance is a better index for determining the functional state of lipid metabolism.

Zilversmit's postulation of postprandial hyper lipidemia as a possible factor for pathogenesis of atherosclerosis aroused interest in determination of postprandial changes in lipid fraction after a meal rich in fat and cholesterol.

DIFFERENT FACTORS REGULATING FAT TOLERANCE

Age has been shown an important factor. Chylomicron count has been shown to rise more after a fat load in subjects more than 50 years as compared to the younger group (Becker et al, 1949). Bernstein et al (1953) observed that the total fats persisted longer in serum after fat loading in older subjects. Body weight and the duration of lipemia were shown to be poorly related (Barritt, 1956).

Nissen (1931) showed that at rest the lipid level of normal subjects increased by 62% after 3 hours of fat meal and the maximum was attained after 4 hours, while at work these figures were 36% at 3 hours.

Smoking has also been shown to affect postprandial hyperlipemia. In habitual smoker, response to a fat meal indicated a lower postprandial rise in serum fat than to non-smoker (Kenttinen and Rajasalmi, 1963). Harder et al (1952) showed that one cigarette/hour caused the chylomicron count to rise in a group of young subjects but not in 2 elderly subjects.

Barritt et al (1956) could not relate significantly between body weight and duration of lipemia, however, it was shown that the fat tolerance rose appreciably after weight reduction was enforced.

REPRODUCIBILITY OF FAT TOLERANCE

Reproducibility of fat tolerance has always been a controversial issue. While Morton (1950) and Osme et al (1957) showed reproducibility of test over a period of six months, Krantz Stewart and Blackburn (1958) found considerable variability in response to the same fat load.

MATERIAL AND METHODS

MATERIAL AND METHODS

The case material for the present study consisted of 31 healthy male and 17 healthy female volunteers of 15-46 years of age. The subjects were randomly selected from among the junior doctors and students of M.L.B. Medical College, healthy attendants of the patients attending OPD and wards of Medical College Hospital, Jhansi and domestic servants.

Informed consent was taken from every case. Detailed history taking, thorough clinical examination and relevant investigation were done to exclude any cause of hyper lipoproteinemia. Detailed dietary history was also elicited to assess the amount of different constituents - fat and cholesterol, protein and carbohydrate in the usual diet. Daily fat consumption (with its type egg, ghee, oil, milk and milk products,-eggs and food additives). Majority of the subjects were consuming less than 300 mg of cholesterol and P/S ratio of the usual diet ranged from 0.40 to 0.55. No female subject of the study was using oral contraceptive at the time of study or 3 months prior to it.

DESIGNS OF TEST DIET

As this study was taken to assess the changes in serum lipid profile after ingesting different amount and types of cholesterol fat diet (and other types of

diets. protein, carbohydrate), 14 different protocols were used.

All the subjects were asked to have their dinner at 6.00 PM on the previous night and not to take anything except water till the next morning. Fasting blood samples were taken at 8 AM in recumbent posture without producing venous stasis (Koerselman et al,1961). After this they were given a test meal which differed in different protocols.

Postprandial blood samples were taken at different time intervals in different protocols. All subjects were confined to bed at the time of test and were not allowed to take anything except water. Five millilitres of blood was collected for each test sample and plasma was separated from the blood within four hours. Following tests were performed in each blood sample.

1. SERUM TOTAL CHOLESTEROL (STC)

Cholesterol estimation was done by one step method of Aybenga and Pileggi (1970) utilising commercial kits supplied by ERBA.

2. SERUM TRIGLYCERIDE(STG)

Serum triglyceride was estimated by acetyl acetone method, utilising kits provided by HI-TECH Laboratories.

3. SERUM HIGH DENSITY LIPOPROTEIN(HDL)

Quantitative estimation of HDL cholesterol in serum was done by kit supplied by ERBACH, using the precipitating method.

4. SERUM LOW DENSITY LIPOPROTEIN(LDL)

This fraction of lipoprotein was estimated by formula given by Friedwald et al. (1972).

LDL = STC - (STG/5 + HDL) mg/dl.

5. SERUM VERY LOW DENSITY LIPOPROTEIN(VLDL)

This too was estimated by above mentioned formula which is valid till STG values are less than 500 mg/dl.

Statistical analysis of the data was done by using different tests of significance (Paired 't' test and student 't' test).

PROTOCOL 1

Number of subjects - 10(6 males and 4 females).

TEST DIET : 100 gm butter smeared over 4 average sized breads and 200 ml of boiled sweetened milk. The provided about 300 mg cholesterol and 95 gm of fat.

Postprandial blood samples were taken at one hour interval upto five hour interval in most of the subjects.

PROTOCOL 2

Number of subjects : 5(4 males and 1 female)

TEST DIET : 50 gm butter smeared over 4 breads with 200 ml of milk. This provided about 160 mg of cholesterol and 55 gm of fat.

Postprandial blood samples were taken at one hour interval upto five hours.

PROTOCOL 3

Number of subjects : 8(4 males and 4 females).

TEST DIET : Single boiled hen egg, supplying about 300 mg of cholesterol and 6 gm of fat.

Postprandial blood samples were taken at one and three hour.

PROTOCOL 4

Number of subjects : 2 (1 male and 1 female).

TEST DIET : 2 boiled eggs, providing about 600 mg cholesterol and 12 gm of fat.

Postprandial samples were taken at one and three hour.

PROTOCOL 5

Subjects : 2 (1 male and 1 female).

TEST DIET : 3 boiled eggs plus 250 ml of milk (The same test diet was used in work done by Arora et al, 1989; when concept of cholesterol tolerance test was proposed).

Postprandial samples were taken at 15 minutes interval upto 1 hour and one sample at 2nd hour.

PROTOCOL 6

Subjects : 2 male subjects.

TEST DIET : 4 boiled eggs, providing about 1200 mg cholesterol and 24 gm fat.

Postprandial samples were taken at 1 hour interval upto 3 hours.

PROTOCOL 7

Number of subjects : 2 male subjects.

TEST DIET : 6 boiled eggs, providing about 1800 mg of cholesterol and 36 gm of fat.

Postprandial samples were taken at one hour interval upto three hours.

PROTOCOL 8

Subjects : 2 (1 male and 1 female).

TEST DIET : 4 eggs albumin providing about 24 gm of fat and 24 gm of protein.

Postprandial samples were taken at one hour interval upto 3 hours.

PROTOCOL 9

Number of subjects : 2(1 male and 1 female).

TEST DIET :75 gm of glucose dissolved in water.

Postprandial samples were taken at 1, 2 and 3 hours.

PROTOCOL 10

Number of subjects : 2 male subjects.

TEST DIET : 50 gm of pure (desi) ghee with 4 breads.

Postprandial samples were taken at first, second, third and fifth postprandial hours.

PROTOCOL 11

Number of subjects : 2 (both males).

TEST DIET : 50 gm of saffola (Kardi) oil with 4 breads.

Postprandial samples were taken at one and three postprandial hours.

PROTOCOL 12

Number of subjects : 1 male subject.

TEST DIET: Alcohol in the form of 100 ml whisky 42.8% v/v, 750 proof.

Postprandial samples were taken at one and three hours.

PROTOCOL 13

Number of subjects : 8(male : female = 1:1).

TEST DIET : 1 gm of crystalline cholesterol dissolved in 200 ml of milk.

Postprandial samples were taken at 1 & 3 hours.

PROTOCOL 14

Number of subjects : 2 (males; these were same subjects which were studied in protocol 5).

These subjects were asked to have 2 eggs plus 200 ml milk daily in their breakfast after single dose 'cholesterol tolerance test'. The second study was conducted after 15 days of first study.

TEST DIET : 3 eggs plus 250 ml of milk.

Two postprandial samples were taken at one and three hours.

For the ease of study, subjects in these protocols were grouped as follows :

GROUP A : Subjects studied under protocol 1 and 2(Test diet in the form of different amounts of butter).

GROUP B : Subjects studied under protocol 3(Test diet in the form of single egg).

GROUP C : Subjects studied under protocols 4,5,6 and 7(Test diet in the form of different amounts of egg cholesterol).

GROUP D : Subjects studied under protocols 10,11, and 12 (Test diet in the form of miscellaneous food articles).

GROUP E : Subjects studied under protocol 13 (Test diet in the form of crystalline cholesterol).

Subjects in protocol 5 and 14 were the same and they were put in group C.

ASSESSMENT OF THE INDIVIDUAL RISK

Individual risk for developing atherosclerosis related disorder was also assessed by studying both - basal lipid lipoprotein profile and test diet induced changes in HDL, LDL and VLDL.

1. Assessment of risk by studying fasting (basal) profile : For this fasting STC and LDL/HDL ratio in each individual was assessed and subjects were categorised in following groups.

a. Low Risk Group

STC level less than 220 mg/dl and
LDL/HDL ratio less than 3.

b. Border Line or Moderate Risk

i. STC level more than 220 mg/dl or
ii. LDL/HDL ratio more than 3, if STC level is less than 220 mg/dl.

c. High Risk

STC level more than 220 mg/dl and
LDL/HDL ratio more than 3.

2. Evaluation of risk after giving test diet was also done : This was based on an arbitrary scale, where definite points were given on a definite change in quantum of LDL, VLDL and HDL. Following was the scheme.

Percentage of rise in basal level of LDL, VLDL & HDL.	Scoring		
	LDL	HDL	VLDL
Upto 5	0	0	0
5 to 15	+ 1	- 1	+0.5
15 to 30	+ 2	- 2	+1
30 to 45	+ 3	- 3	+1.5
45 to 60	+ 4	- 4	+2
Percentage of fall in basal level in LDL, VLDL & HDL			
Upto 5	0	0	0
5 to 15	- 1	+ 1	-0.5
15 to 30	- 2	+ 2	-1.0
30 to 45	- 3	+ 3	-1.5
45 to 60	- 4	+ 4	-2

OBSERVATIONS

OBSERVATIONS

The present work was conducted in 31 male and 17 female subjects of 15-60 years of age. They were divided into following 5 groups as per protocol of study as has already been detailed in material and methods.

GROUP A

It consisted of 10 male and 5 female subjects in age group 15-46 years with mean age of 27.7±8.4 years and mean weight of 62.4±18.0 kg. Their some general particulars are shown in table 1.

GROUP B

It comprised of 8 subjects (male : female = 1:1) in age group of 20-34 years with mean age of 27.1±5.9 years and mean weight of 52.2±8.0 kg. Their other general particulars are depicted in table 1.

GROUP C

It comprised of 8 subjects (6 males and 2 females) in age group of 19-42 years with mean age of 30.8±7.1 years and mean weight of 57.1±7.3 kg. Their general particulars are given in table 2.

GROUP D

It comprised of 9 subjects (7 male and 2 female) in age group of 15-60 years with mean age of 32.5±14 years and mean weight of 55.3±9.2 kg. Their general

particulars are given in table 2.

GROUP I

It consisted of 4 male and 4 female subjects in age group of 20-35 years with mean age of 29.8±4.5 years and mean weight of 54±7.8 kg. Their general particulars are depicted in table 3.

TABLE 1 : Showing general particulars of subjects of group A and B.

Characteristics	Group A(n=15) No.	(%)	Group B(n=8) No.	(%)
OCCUPATION				
Junior Doctors	2	13.3		
Students	5	33.3	2	25.0
Manual workers(Labourer, domestic servants, farmers and others)	2	13.3	1	12.5
House wives	4	26.6	3	37.5
Businessmen	1	6.6	2	25.0
Executives	1	6.6	-	-
PHYSICAL ACTIVITY				
Sedentary	8	53.2	5	62.5
Moderate	4	26.6	3	37.5
Heavy	3	20.0	-	-
DIETARY HABITS				
Vegetarian	10	67.0	5	62.5
Non-vegetarian	5	33.0	3	37.5
SMOKING				
Smoker (7to cigarette/day)	2	13.0	2	25.0
Nonsmoker/Occasional	13	87.0	6	75.0
FAT TOLERANCE				
Low	6	40.0	3	37.5
Moderate	8	53.2	5	62.5
Heavy	1	6.6	-	-

TABLE 2 : Showing general characteristics of subjects of group C and D.

Characteristics	Group C(n=8) No.	(%)	Group D (n=9) No.	(%)
OCCUPATION				
Student	1	12.5	1	11.1
Manual workers	3	37.5	2	22.2
Bussinessman	1	12.5	3	33.3
Housewives	2	25.0	2	22.2
Army personnal	1	12.5	-	-
Government servant	-	-	-	-
PHYSICAL ACTIVITY				
Sedentary	4	50.0	5	55.6
Moderate	1	12.5	3	33.3
Heavy	3	37.5	1	11.1
DIETARY HABIT				
Vegetarian	3	37.5	5	55.6
Non-vegetarian	5	62.5	4	44.4
SMOKING				
Smoker ($\geq$10 cig./day)	4	50.0	5	55.6
Nonsmokers or $\leq$10 cig./day	4	50.0	4	44.4
FAT CONSUMPTION				
Low	4	50.0	7	77.8
Moderate	2	25.0	2	22.2
High	2	25.0	-	-

TABLE 3 : Showing general characteristics of subjects of group C.

Characteristics	Group C (n = 8) No.	(%)
OCCUPATION		
Businessmen	3	37.5
Labourer	1	12.5
Housewives	4	50.0
PHYSICAL ACTIVITY		
Sedentary	5	62.5
Moderate	2	25.0
Heavy	1	12.5
DIETARY HABIT		
Vegetarians	5	62.5
Non-vegetarians	3	37.5
SMOKING		
Smokers	1	12.5
Nonsmokers	7	87.5
FAT CONSUMPTION		
Low	6	75.0
Moderate	2	25.0
High	-	-

TEST DIET INDUCED CHANGES IN SERUM TOTAL CHOLESTEROL(STC) LEVEL IN DIFFERENT STUDY GROUPS

TABLE 4 : Showing relation of different parameters with fasting STC in present study(Mean±S.D. mg/dl).

Parameters		STC
1. SEX : Male (n=31)		198.6±31.4
Female (n=17)		195.6±20.2
t = 0.437 P > 0.05		
2. PHYSICAL ACTIVITY		
Sedentary (n=27)	(I)	200.8±23.2
Moderate (n=13)	(II)	197.2±32.9
Heavy (n=8)	(III)	170.8±21.1
I : III, t = 1.986, P < 0.05		
3. FAT CONSUMPTIONS		
Low (n=26)	(I)	187.1±20.3
Moderate (n=19)	(II)	201.7±27.6
High (n=3)	(III)	236.8±34.7
I : II, t = 2.021, P < 0.05		
I : III, t = 3.301, P < 0.01		
4. SMOKING		
Nonsmokers (n=34)	(I)	192.8±30.7
Smokers (n=14)	(II)	202.2±40.2
I : II, t = 0.785; P > 0.05		

All subjects except one (2.08%) in the present study had fasting STC level within 240 mg/dl. 16(33.3%) had their levels in the range of 210-240 mg/dl while

remaining 31 (44.6%) had their fasting level below 210 mg/dl. The mean fasting level in males (n=31) was 195.6±31.4 mg/dl. This level was almost similar to that of females (n=17) which was 195.6±20.2 mg/dl. Mean STC in sedentary persons was 200.8±23.2 mg/dl while in moderately active it was 197.2±32.9 mg/dl. Persons, who used to do heavy exercise had significantly low level of STC (170.8±21.1 mg/dl. P $<$0.05). There was no significant effect of smoking on fasting STC in present study. Amount of fat intake had significant impact on STC levels, while no significant difference could be observed between vegetarians and nonvegetarians (Table 4).

CHANGES IN GROUP A

Protocol 1

In protocol 1, when test diet was given, there was rise in STC at first hour. This further increased at second hour in most of the subjects. This level started showing fall and was near the basal level at about 5 hour (Table 5).

In 8(80%) subjects of this group there was a peak rise at second postprandial hour. In one subject peak level was attained at first hour while one subject showed a fall from basal level. The maximum magnitude of rise of 22 mg/dl (17% of basal) and of fall of 19mg/dl (8.2% of basal) was observed. The range of rise was between 5 to 12.9 percent of basal value.

The rise was more marked in subjects having comparatively low STC level. The rise was more prominent in females. Age could not be related with the magnitude of rise in this group, basically because most of the subjects in this group were young (15.35 years). No correlation could be observed between magnitude of rise and dietary habit, amount of daily fat intake, smoking habit, body weight and type of life style.

Protocol 2

The mean STC in this subgroup was 185.6±33.9 mg/dl. After ingestion of test diet this started rising and attained to peak at 2 hour in all but one subject. In most subjects it returned to basal level at 5 hours (Table 5). The maximum quantum of rise of 28 mg/dl (15.3% of basal) was observed, in 2 subjects. The range of rise was between 0 to 15 percent of basal value. One subject showed fall of 16 mg/dl (7.8% of basal).

TABLE 5 : Showing changes in STC in group A subjects.

Protocol	Fasting(I)	2 hour after test diet(II)	5 hour after test diet(III)
1 (n=10)	189.1±24.1	216.1±18.2	204.3±19.8
	I : II, t = 2.812,	P <0.02	
	I : III, t = 2.136,	P <0.05	
2 (n=5)	185.6±33.9	196.0±30.8	192.0±30.4
	I : II, t = 1.876,	P >0.05	
	I : III, t = 1.636,	P >0.05	

STC progressively increased with age in this group. The magnitude of rise in STC was less marked in subjects following this protocol, in comparison to previous one, where, just double amount of cholesterol fat was used.

CHANGES IN GROUP B

Protocol 3

Mean fasting STC was 187.0±27.1 mg/dl in this group. When test diet was given it fell to 172.2±21.8 mg/dl at first hour. The value was near the basal value at 3 hour. This fall in STC was statistically significant (P $<$0.05) (Table 6).

The mean fasting STC in males (177.7±28 mg/dl) was insignificantly lower than females (198±21.9 mg/dl). After ingestion of test diet 6(75%) subjects showed fall in STC at 1 hour. Two (25%) subjects showed an insignificant rise at first hour. Maximum magnitude of fall of 26 mg/dl (11.5% of basal) and rise of 13 mg/dl (7.9% of basal) was observed.

TABLE 6 : Showing changes in STC in group B(Mean±SD mg/dl)

Protocol	Fasting (I)	2 hour after test diet(II)	3 hour after test diet(III)
3 (n=8)	187.0±27.1	172.2±21.8	183.3±23.7
	I : II, t = 2.011, P $<$0.05		

CHANGES IN GROUP C

In this group 4 different protocols were used in which different amount of egg cholesterol was used.

Protocol 4

When 2 eggs diet was given to 2 subjects two different feeding behaviour was noted. In 1 subject(male) STC increased from 225 mg/dl to 238 mg/dl at first hour and fell to 236 mg/dl at third hour. Thus there was rise of 5.4% of the basal at first hour.

In second subject (female) STC fell from basal value by 14 mg/dl (7.7% of basal) at first hour. It further fell by 7 mg/dl at 3rd hour.

Protocol 5

This protocol used the same test diet as proposed by Arora et al (1968). The subjects following this protocol were one 32 years female and another 35 years old male subject.

In first subject STC level increased from 236 to 250 mg/dl - a rise of 14 mg/dl which accounted for 6% of basal value. Third hour sample was similar to first hour sample. Second subject showed a fall of 20 mg/dl (8.6% of basal).

In both these subjects samples were collected at 15 minutes, interval upto 1 hour to know the onset of rise or fall of STC when test diet is given. The study of STC in different 15 minutes sample showed that if the

STC is destined to increased at first hour, the rise becomes apparent right from 15 minutes,sample and if it is destined to fall at first hour, this fall is there from begining. Apart from sex difference, both these subjects had similar life style and daily fat intake.

Protocol 6

When test diet consisting of 4 eggs was given to 2 subjects, both of them showed fall in STC at first hour which became more or less near the basal value at 3rd hour. In first subject STC was high (200 mg/dl) which decreased to 276 mg/dl at first hour - a meagre 1.6% fall. In second subject STC was normal 200 mg/dl. Here the fall of 7% of basal value was observed. Both the subjects were smokers, non vegetarians and used to consume moderate amount of fat per day.

Protocol 7

Two male subjects were given a test diet consisting of 6 eggs. In both the subjects level fell at first postprandial hour. The magnitude of fall were 14 mg/dl (7.7% of basal)and 18 mg/dl(8.2% of basal) respectively. Third postprandial hour STC levels were close to fasting.

Following important observations were made by studying different protocols in this group :

1. The changes in STC are little influenced by amount of egg yolk cholesterol in test diet.

2. Subjects who had comparatively higher basal STC showed little change after feeding while those with normal STC levels, showed more marked changes (rise or fall) after feeding.
3. Subjects in whom STC is destined to fall at one hour, it starts falling from very begining. Same is true for rise of STC after feeding test diet.
4. The changes in STC after feeding are not very marked excluding few individual cases.

When the data of these different protocols were pooled, these changes reflected statistically insignificant. (Table 7).

TABLE 7 : Showing effect of different amount of cholesterol on STC at 1 and 3 hour (Mean±S.D. mg/dl).

Fasting (I)	1 hour after test diet(II)	3 hour after test diet(III)
218.6±30.7	211.5±37.5	220.0±34.0
I : II,	t = 1.327,	P 70.05
I : III,	t = 0.896,	P 70.05
II : III,	t = 1.398,	P 70.05

CHANGES IN GROUP D

In this group, 5 different protocols were followed, in which different unconventional dietary items were used in test diets. These dietary items had nil or insignificant amount of cholesterol.

Protocol 8

When test diet consisting of 4 egg albumin (egg yolk removed) was used in 2 subjects, one subject(male)

showed increase in STC level at first postprandial hour which further increased insignificantly at second postprandial hour. This value showed decline at 3 hour but was still higher than the basal value. The rise was 8.4% of fasting balue.

In second subject (female) STC increased at first hour (6.1% of basal level) and then showed decline at second and third hour. The third hour value was approaching the basal value. The quantum of rise in both the subjects was less than 10% of the basal value.

Protocol 9

Test diet in the form of 75 g glucose dissolved in 200 ml of water was given to one young female subject aged 20 years and another male aged 55 years. In first subject there was rise in STC (6% of basal) while in other there was fall in STC (4% of basal).

Protocol 10

In this subgroup test diet was given to 2 cases. Both of them showed rise in STC with peak level at 2 hour in first case and 3rd hour in second case. The quantum of rise in first case was 17.1% of basal, while in second case it was 13% of basal level.

Protocol 11

When 2 male subjects were given 50 gm of saffola oil with 4 breads STC increased in both the cases by 7%

of basal value and 8.5% of basal value respectively. The level reached near basal value at 3 hour.

Protocol 12

In this protocol a single subject was given a diet based on 100 ml alcohol. The STC increased by very meagre margin (4% of basal). This subject had been taking alcohol in moderate amount for last 10-12 hour. The fasting STC was 173 mg/dl.

In studying different protocols, where unconventional test diet viz. pure ghee, saffola oil, egg albumin, glucose and alcohol were given insignificant changes in STC were observed.

CHANGES IN GROUP B

The mean STC in this group of subjects was 193.2 ±17.0 mg/dl. It increased to 210.0±30.1 mg/dl after ingestion of test diet (1 g crystalline cholesterol plus 200 ml milk) at first postprandial hour. The value further increased to 216.75±29.7 mg/dl at 2rd hour. The changes were found to be statistically significant(Table 8).

Out of 8 subjects, 6(75%) showed rise in STC at first hour, while 2(25%) showed a fall from fasting value. Maximum quantum of rise of 34 mg/dl (19.7%)of basal) and fall of 18 mg/dl (10.7% of basal) was observed. Subjects having comparatively low fasting STC showed more marked rise in STC after test diet feeding. The range of rise was 8.3 to 19.7% of basal value.

TABLE 8 : Showing changes in group E subjects in STC levels (Mean±S.D. mg/dl).

Fasting(I)	1 hr after test load(II)	3 hr after test diet(III)
193.2±17.0	210.0±30.1	216.0±23.7
I : II,	t = 1.996	P $<$ 0.05
I : III,	t = 1.489	P $<$ 0.05

EFFECT OF PROLONGED FEEDING ON STC

Two subjects who were studied under protocol 5 were asked to take 2 eggs plus 250 ml milk daily in their breakfast for 15 days. Fasting sample when taken on 16th day showed following changes :

	Fasting STC (study 1)	After 15 days of feeding (study 2)
1.	236.00 mg/dl	230.00 mg/dl
2.	230.00 mg/dl	240.00 mg/dl

These changes were statistically insignificant and showed that this diet was not sufficient to cause any change in STC even after prolonged feeding for 15 days.

REPRODUCIBILITY OF TOLERANCE TEST

These 2 subjects were again given the same test diet (3 eggs plus 250 ml milk) on day 16 to assess the reproducibility of tolerance test. It was seen that though absolute values differ considerably at first and third hour, the feeding behaviour of both the individuals remained the same. In first subject, there was rise in STC at 1 hour in both the studies while in

second subject there was fall at 1 hour. The quantum of rise and fall also varied considerably.

CHANGES IN HIGH DENSITY LIPOPROTEIN (HDL) IN DIFFERENT STUDY GROUPS

The mean HDL level in males was 50.6±8.9 mg/dl while in females it was 56.4±6.8 mg/dl. No statistically significant difference could be showed between HDL level and type of diet, dietary fat intake, weight smoking and type of life style.

CHANGES IN GROUP A

Protocol 1

The mean fasting HDL in 10 subjects of this group who were given a test diet of 100 gm butter and 4 breads was 51.1±6.6 mg/dl. It increased significantly to 58.1±8.4 mg/dl at 2nd postprandial hour and then showed a fall at 5th hour. The fifth hour value was 55.2±8.09 mg/dl when compared to fasting and second hour value this difference was statistically insignificant. 9(90%) subjects showed rise after test diet (Table 9) with peak value occurring either at first postprandial hour (44.5% of cases) or at 2nd hour (55.5% of cases). The value reached basal fasting value at 5th hour in most of subjects. The maximum magnitude of rise of 20 mg/dl (35% of basal) and fall of 11 mg/dl (19% of basal) was observed. No significant correlation could be made with fasting HDL and diet induced changes in HDL with that of age, sex, body weight and dietary habits.

Protocol 2

The mean fasting HDL in this group was 44.2± 11.0 mg/dl. This value increased to 52.4±11.5 mg/dl at 2nd hour and fell to 50.0±10.3 mg/dl at 3th hour. All the 5 subjects showed rise after intake of test diet. In 4 subjects(80%) peak was observed at 2 hour, while in one subject(20%) this was observed at first hour(Table 9).

TABLE 9 : Changes in HDL in cases of group A. (Mean ± S.D. mg/dl).

Protocol	Fasting(I)	1 hour after test diet(II)	2 hour after test diet(III)
1	51.1±6.6	56.1±8.5	55.2±8.09
	I : II,	t = 2.013	P <0.05
	II : III,	t = 1.266	P >0.05
	I : III,	t = 1.788	P >0.05
2	44.2±11.0	52.4±11.5	50.0±10.35
	I : II,	t = 1.663	P >0.05
	I : III,		P >0.05
	II : III,		P >0.05

CHANGES IN GROUP B

The mean fasting HDL in this group was 49.1 ±7.6 mg/dl. It increased insignificantly to 49.5±8.1 mg/dl at first hour (P >0.05). It fell to 47.5±7.6 mg/dl at third postprandial hour (Table 10). In 4 subjects (50%), there was rise in serum HDL at first postprandial hour while remaining 4 subjects showed fall. Maximum quantum of rise of 8 mg/dl was observed.

TABLE 10 : Showing changes in HDL in group B.
(Mean ± S.D. mg/dl.

Fasting	1 hour after test diet(II)	3 hour after test diet(III)
49.1±7.6	49.5±8.1	49.1±7.6
I : II	p 70.05	
I : III	p 70.05	
II : III	p 70.05	

CHANGES IN GROUP C

Protocol 4

When test diet of 2 eggs was given in 2 subjects HDL increased in both the cases by 4 mg/dl(7.1% of basal) and 6 mg/dl (10% of basal) respectively.

Protocol 5

After ingestion of test diet HDL increased in both the subjects by 4 mg/dl (11.1% and 8% of basal value respectively).

Protocol 6

HDL increased by 6 mg/dl (10.7% of basal) at first hour in one subject, while it fall by 8 mg/dl (14.2% of basal) in the other.

Protocol 7

HDL increased in both the cases by 8 mg/dl and 14 mg/dl (13.7% and 28% of basal) respectively after ingestion of test diet.

Thus there was little change in HDL level when different amount of egg cholesterol was given except in few individual cases. The amount too has little relation with changes in HDL level.

CHANGES IN GROUP D

When different types of test diets(vide supra) were given, this group of subjects showed little insignificant rise with almost all types of diet. Maximum magnitutde of rise was seen when test diet based on ghee (pure) was used. It was 8 mg/dl at 2nd hour (which accounted for 17.3% of basal value). Fall in HDL level was observed when alcohol was given. It fell by 10 mg/dl (16.6% of basal).

CHANGES IN GROUP E

The mean fasting HDL was 47.3±10.1 mg/dl. It increased to 59.8±11.8 mg/dl at first postprandial hour. The value at 3rd hour was almost similar(55.2±9.7 mg/dl) when compared with the fasting value. These differences were found to be statistically insignificant (Table 11).

TABLE 11 : Showing changes in HDL in group E. (Mean ± S.D. mg/dl).

Fasting(I)	1 hour after test diet(II)	3 hour after test diet(III)
47.3±10.1	55.8±11.8	55.2±9.7
I : II	P > 0.05	
I : III	P > 0.05	
II : III	P > 0.05	

EFFECT OF PROLONGED FEEDING ON HDL

The HDL level after 15 days of feeding was slightly changed.

	Fasting HDL Levels (mg/dl)	
	Prior to prolonged feeding	After prolonged feeding
1.	36	40
2.	62	56

REPRODUCIBILITY OF TEST

In both the subjects there was similar change in HDL in first study. It increased by 4 mg/dl(11%) in first subjects, while it increased by 8 mg/dl (11% over basal) in second. Study-2 showed the HDL level, too, increased in both the cases, though the quantum of this rise was variable.

CHANGES IN SERUM TRIGLYCERIDE (STG)

The mean serum triglyceride in males was 166.2±24.8 mg/dl which significantly higher than the females (150.4±22.6 mg/dl, P $<$0.05). The smokers(n=14) had mean fasting triglyceride level of 160.16±25.33 mg/dl, which was similar in non smokers (158.1±14.4 mg/dl). Physical activity and intake of fat influenced the STG level in the present study, but type of diet intake (vegetarian or non vegetarian) had little influence on it (Table 12).

TABLE 12 : Showing relation of different parameters with fasting STG (Mean ±S.D. mg/dl).

Parameters		STG
Physical activity		
1. Sedentary (n=27)	(I)	170.4±22.8
2. Moderately active (n=18)	(II)	166.8±18.9
3. Heavy (n=8)	(III)	150.8±26.8
I : III, t = 1.969,		P < 0.05
Fat Intake		
1. Low (n=28)	(I)	168.5±22.6
2. Moderate (n=19)	(II)	156.5±28.8
3. High (n=3)	(III)	158.7±18.2
No statistical significance was observed		
Type of diet Intake		
1. Vegetarian (n=28)	(I)	162.5±25.3
2. Non vegetarian (n=20)	(II)	168.5±18.4

CHANGES IN GROUP A

Protocol 1

The mean fasting STG of 10 subjects following this protocol was 157.6±19.4 mg/dl, when test diet was given this value increased to 161.6±14.8 mg/dl at 1st postprandial hour and fell back to near basal value (159.6±16.6 mg/dl) at 9th postprandial hour (Table 13). These differences were not statistically significant. Like STC, STG level peaked at 2 hour in 70% of cases.

The peak was seen at first hour in 20% of cases while in one subject (10%) there was fall of STG level after feeding. The maximum magnitude of rise of 24 mg/dl (14.6% of basal value) was seen.

Protocol 2

Five subjects in this subgroup had mean fasting STG value of 153.2±26.9 mg/dl. It increased to 165.2± 27.8 mg/dl at 2 hour and fall back to near basal level at 5th hour. These differences were statistically insignificant. The maximum quantum of rise of 20 mg/dl (11.3% of basal) was seen (Table 13).

TABLE 13 : Showing changes in STG level in group A (Mean ±S.D. mg/dl)

Protocol	Fasting(I)	2 hour after test diet(II)	5 hour after test diet(III)
1	157.8±19.4	161.0±14.8	159.8±16.6
	I : II	t = 0.673,	P >0.05
	I : III	t = 0.590,	P >0.05
	II : III	t = 0.787,	P >0.05
2	153.2±26.9	165.2±27.8	157.2±22.6

No statistical significance between these changes was observed.

CHANGES IN GROUP B

Subjects of this group had mean fasting STG level 141.7±20.1 mg/dl. This value was 140.7±18.2 mg/dl at first hour after taking test diet and 139.0±17.6mg/dl at third postprandial hour (Table 14). These changes

were statistically insignificant, when paired 't' test was applied.

STG fell in 4 subjects (50%), while it increased in remaining 50% subjects at first hour. The maximum quantum of rise of 10 mg/dl (7.5% of basal) and fall of 12 mg/dl (7.7% of basal) was seen at first hour after taking test diet.

TABLE 14 : Showing changes in STG level in group B. (Mean ± S.D. mg/dl).

Fasting(I)	1 hour after test diet(II)	3 hour after test diet(III)
141.75±20.1	140.75±14.2	139.0±17.69
I : II,	t = 0.437	
I : III,	t = 0.461	
II : III,	t = 0.369	

CHANGES IN GROUP C

Protocol 4

STG level increased in both the subjects at first postprandial hour by 12 mg/dl (6.5% of basal) and 4 mg/dl (2.5% of basal). This further increased at 3rd hour in first subject, while it was nearr basal level in second subject.

Protocol 5

STG level increased in first subject by 14 mg/dl (8% of basal) at first postprandial hour, while it decreased by 14 mg/dl (6.6% of basal) in second subject. Third hour value was similar to basal value in both of the subjects.

Earlier work done by Arora et al (1968)

the subjects.

Earlier work done by Arora et al (1988) in this department showed a fall in LDL and STG level in majority of the healthy subjects when same test diet was given.

Protocol 6

When 2 subjects of this subgroup were given a test diet similar pattern was seen while one subject showed a fall of 18 mg/dl (9% of basal), other subject showed rise of 8 mg/dl (5% of basal value).

Protocol 7

In this group both the subjects showed rise in STG level at first hour. This rise was 20 mg/dl (14.7% of basal value) in first subject, it was just 6 mg/dl (4% of basal value) in second subject. In both subjects third hour value was similar to basal values.

When data of this group were pooled together it was seen that in 6 subjects (75%), there was rise in STG level at first hour after receiving different amount of egg yolk diet, while 2 subjects (25%) showed fall.

CHANGES IN GROUP B

Protocol 8

When test diet consisting of egg albumin was given to 2 subjects of this group, one showed a rise

of 6 mg/dl (3.3% of basal) and other showed a fall of 5 mg/dl (3% of basal).

Protocol 9

Similar trend as above was observed. A fall of 8 mg/dl (4.2% below basal level) and rise of 11 mg/dl (6.7% of basal value) was seen at first hour after taking test diet.

Protocol 10

In this subgroup STG level increased in both subjects by 12 mg/dl (10% of basal) and 18 mg/dl (12% of basal) respectively at first hour of feeding. At third hour these values were near basal value.

Protocol 11

In this subgroup, also, STG level increased at first postprandial hour. The rise was 10 mg/dl in both the subjects, which accounted 5.9% and 5.9% of basal value respectively.

Protocol 12

STG level increased by a meagre margin of 6 mg/dl (13% of basal value) when subjects was given diet based on alcohol.

CHANGES IN GROUP B

The mean fasting STG of this group subject was 164.8±17.5 mg/dl. It increased significantly to

176.5±22.2 mg/dl at first hour. This value fell to 170.7±26.7 mg/dl at 3rd hour (Table 15). The maximum magnitude of rise was 18 mg/dl (12% of basal value).

TABLE 15 : Showing changes in STG level in group E. (Mean ± S.D. mg/dl).

Fasting (I)	1 hour after test diet(II)	3 hour after test diet(III)
164.8±17.8	176.8±22.8	170.7±26.7
I : II.	t = 2.013	P < 0.05

EFFECT OF PROLONGED FEEDING ON STG LEVEL

Effect of prolonged feeding on STG was observed in 2 subjects. This feeding increased STG level in both the cases.

	Fasting STG levels (mg/dl)	
	Prior to prolonged feeding	After prolonged feeding
1.	176	190
2.	210	216

REPRODUCIBILITY OF FEEDING BEHAVIOUR

The STG levels in prior study increased after feeding in one subject, while it decreased in other. this time, too, the same behaviour pattern was duplicated though absolute values differed.

CHANGES IN VLDL

As VLDL levels were calculated from STG levels. the changes in this parameter ran exactly parallel to STG changes.

CHANGES IN LOW DENSITY LIPOPROTEIN (LDL)

TABLE 16 : Showing changes in LDL with different parameters (Mean ± S.D. mg/dl).

Parameters		LDL
1. SEX :		
Male		117.2±25.6
Female		114.1±22.5
	P > 0.05	
2. DIETARY HABIT		
Nonvegetarians		118.3±33.6
Vegetarians		98.5±19.8
	t = 2.647, P < 0.05	
3. SMOKING HABIT		
Smokers		118.3±27.2
Non smokers		110.0±24.0
	P > 0.05	
4. PHYSICAL ACTIVITY		
Sedentary	(I)	120.6±30.6
Moderate	(II)	101.7±29.5
Active	(III)	104.3±33.2
I : II,	t = 1.902, P < 0.05	
5. DAILY FAT INTAKE		
Low	(I)	106.2±30.4
Moderate	(II)	116.7±23.2
High	(III)	132.7±26.6
I : II,	t = 1.823, P > 0.05	
I : III,	t = 3.968, P < 0.05	
II : III,	t = 2.403, P < 0.05	

Mean serum LDL was 117.2±26.6 mg/dl in males subjects in this study, which was insignificantly higher (P 70.05) than females (114.1±22.5 mg/dl). Vegetarians had significantly lower level than non vegetarians. There was no significant change between smoker and non smoker. Physical activity did exert the effect on LDL level. The isignificant effect was observed between sedentary persons and moderately active persons, while active persons had almost same level as possessed by moderately active persons. Amount of fat intake also influenced the serum LDL level. It was higher in high fat consumers, while low in moderate and low fat consumers (Table 16).

CHANGES IN GROUP A

Protocol 1

Fasting LDL level in this group of subjects was 113.8±14.1 mg/dl which increased after feeding. The peak was attained at first hour in 3 (30%) subjects, while it was at second hour in 5 subjects (50%). One subject showed fall at first and second hour, while in other there was virtually no change in first and second hour. The mean value at second hour was 123.9±14.2 mg/dl. The value at fifth hour was almost at basal level 117.1±14 mg/dl. These changes at second hour was found to be statistically significant (Table 17). The maximum magnitude of rise of 22 mg/dl (20% of basal) was observed

while one subject showed a fall of 19 mg/dl (23% of basal value).

Protocol 2

Five subjects who were fed 50 gm of butter showed two types of responses :

1. In 3(60%) subjects LDL level fell at first hour.
2. In 2(40%) subjects LDL level increased at 1 hour.

The mean LDL value in this group was 110.3± 38.9 mg/dl. At hour this value fell to 107.3±47.0 and at fifth hour, it was similar to fasting value (111.3± 42.6 mg/dl). These values were not statistically significant (Table 17). The maximum quantum of rise 21 mg/dl (34% of basal value) and maximum quantum of fall was 22 mg/dl (29% of basal value).

TABLE 17 : Showing changes in serum LDL level in group A (Mean ± S.D. mg/dl).

Protocol	Fasting(I)	1 hour after test diet(II)	3 hour after test diet(III)
1	113.8±14.1	123.9±14.2	117.3±14.0
	I : II, t = 2.031,		P <0.05
2	110.3±38.3	107.3±47.0	111.3±42.6

CHANGES IN GROUP B

Protocol 1

Eight subjects of this group were fed a single egg test diet. The fasting LDL level in this subject

was 110.4±20.9 mg/dl. This level fell to 94.85±20.9 mg/dl at first postprandial hour. The level at 3 hour was near basal level (Table 18). Six(75%) subjects showed fall at 1 hour, while 2(25%) subjects showed rise at first hour. Maximum fall of 36 mg/dl (42% of basal value) and rise of 15 mg/dl (12.7%) of basal value) was seen.

TABLE 18 : Showing changes in LDL level in group B. (Mean ± S.D. mg/dl).

Fasting(I)	1 hour after test diet(II)	3 hour after test diet(III)
110.4±20.9	94.85±20.9	108.0±23.6
I : II, t = 2.132, P < 0.05		

CHANGES IN GROUP C

Protocol 4

The LDL level in one subject increased by 6 mg/dl (8%)of basal value), while it fell by 19 mg/dl (16% of basal) in other subject in 1 hour.

Protocol 5

The same pattern was observed in this subgroup also. Rise of 8 mg/dl (13% of basal) and fall of 23mg/dl (26% of basal) respectively in two subjects.

Protocol 6

Same pattern was seen here also with one subject showing rise in LDL at 1 hour (16% of basal) and other showing fall of 22% of basal at first hour.

Protocol 7

In this subgroup both the subjects showed a fall of LDL at 1 hour. This fall was 26 mg/dl (24% of basal) and 23 mg/dl (46% of basal) respectively.

CHANGES IN GROUP B

In this group very low variation in level of LDL was seen except in protocol 10, where subjects were fed 50 gm pure ghee with breads. Here one subject showed a rise of 19 mg/dl (19% of basal) at 3rd hour. Similarly drastic rise was also observed in a subject following protocol 11 (Taking diet based on Saffola oil) where a rise of 20 mg/dl, was seen. Of subject who consumed alcohol based diet showed a peak at 2 hour with rise of 22 mg/dl (32% of basal value).

CHANGES IN GROUP E

The mean fasting LDL in this group of subjects was 112.9±18.6 mg/dl. It increased to 118.9±32.8 mg/dl at second hour while it further increased to 120.15±27.5 mg/dl at fifth hour (Table 19). In 6(75%) subjects there was rise in LDL level after feeding the test diet, while in 2(25%) cases there was a fall in LDL level after the ingestion of test diet. The maximum quantum of rise of 33.8 mg/dl (25% of basal value) was seen.

TABLE 19 : Showing changes in serum LDL in group E. (Mean ± S.D. mg/dl).

Fasting(I)	2 hour after test diet(II)	5 hour after test diet(III)
112.9±18.6	118.9±32.8	128.15±27.5

I & III, t = 1.996, P < 0.05

CHANGES IN LDL/HDL RATIO

TABLE 20 : Showing relation of LDL/HDL ratio with different parameters in present study.

Parameters		LDL/HDL Ratio
SEX :	Male	2.2±0.6
	Female	2.4±0.8
SMOKING		
	Smokers	2.1±0.6
	Non smokers	2.3±0.7
DIETARY HABIT		
	Vegetarians	2.3±0.5
	Non vegetarians	2.4±0.9
PHYSICAL ACTIVITY		
	Sedentary	2.4±0.6
	Moderate	1.8±0.4
	Heavy	1.9±0.9
FAT INTAKE		
	Low	2.01±0.5
	Moderate	2.1±0.7
	High	3.1±0.9

These relations were statistically insignificant.

The mean LDL/HDL ratio in fasting stage was 2.2±0.6 in male subjects of this study. Female subjects of this study had similar ratio of 2.4±0.8. The relation of LDL/HDL ratio in fasting stage with different parameters have been shown in table 20.

CHANGES IN GROUP A

Protocol 1

The mean basal ratio was 2.1±0.28 in this group. It became 2.1±0.4 at second hour and 2.1±0.4 at fifth hour. There was no statistical significant.

Protocol 2

The mean basal ratio in 5 subjects of this group was 2.7±1.1. It decreased after feeding and became 2.2±1.1 at second hour. The value at fifth hour was similar to second hour value. These changes were not statistically significant.

CHANGES IN OTHER GROUPS

There was no statistical significance in LDL/HDL ratio in any group after feeding. Fasting ratio and changes in ratio have been shown under assessment of individual risk.

Determination of individual risk for atherogenic complications.

Case No.		LIPID PROFILE					RISK ON THE BASIS OF FASTING LIPID LIPOPROTEIN PROFILE	LIPID RISK BY CHOLESTEROL FEEDING RESULTS (SCORING SYSTEM ON THE BASIS OF ARBITRARY SCALE
		STC	HDL	LDL	VLDL	LDL/HDL		
1	2	3	4	5	6	6	8	9
1.	I	257.0	56.0	116.0	33.0	2.1	The LDL/HDL ratio = 2.1	Test diet induced changes in LDL, HDL & VLDL are when scored on scale described under material and methods. Total score = -1.5, Indeterminate lipid risk.
2.	II	237.0	76.0	123.0	37.5	1.6	Fasting STC = 257mg/dl	
	III	222.0	55.0	133.0	33.6	2.4	Low lipid risk for atherogenesis.	
3.	I	225.0	52.0	142.0	31.0	2.7	LDL/HDL ratio = 2.7	Total score = 0
	II	247.0	56.0	159.0	32.0	2.8	Fasting STC = 225 mg/dl	Indeterminate lipid risk for atherogenesis.
	III	212.0	58.0	131.0	30.0	2.6	Moderate or borderline lipid risk for atherogenesis.	
3.	I	219.0	56.0	123.0	39.0	2.1	LDL/HDL ratio = 2.1	Total score = +1
	II	212.0	60.0	104.0	38.0	1.7	Fasting STC = 219 mg/dl	Increased lipid risk on short term feeding.
	III	236.0	56.0	141.0	38.0	2.5	Low lipid risk (less prone for atherosclerosis related complications)	
4.	I	162.0	38.0	98.0	26.0	2.5	LDL/HDL ratio = 2.5	Total score = +1 High lipid risk for atherogenic complications.
	II	186.0	48.0	108.0	30.0	2.2	Fasting STC = 162 mg/dl	
	III	168.0	35.0	102.0	28.0	2.9	Low lipid risk.	

1	2	3	4	5	6	7	8	9
5.	I	218.0	56.0	128.0	33.0	2.2	LDL/HDL ratio = 2.2	Total score = +2
	II	212.0	47.0	130.0	34.0	2.7	Fasting STC = 218 mg/dl	Inference - high risk
	III	210.0	56.0	120.0	34.0	2.1	Low lipid risk.	on feeding HCFD.
6.	I	176.0	45.0	102.0	28.0	2.2	LDL/HDL ratio = 2.2	Total score = - 0.5
	II	200.0	56.0	113.0	30.8	2.0	Fasting STC = 176 mg/dl	Inference = Indeterminate lipid risk.
	III	182.0	54.0	98.0	30.0	1.8	Low lipid risk for atherogenesis.	
7.	I	178.0	44.0	108.0	26.0	2.4	LDL/HDL ratio = 2.4	Total score = 1.5
	II	210.0	60.0	122.0	28.0	2.03	Fasting STC = 178 mg/dl	Indeterminate lipid
	III	200.0	60.0	113.0	27.0	1.8	Low lipid risk.	risk .
8.	I	289.0	58.0	117.0	34.0	2.01	LDL/HDL ratio = 2.01	Total score = + 1.5
	II	222.0	60.0	125.0	36.0	2.08	Fasting STC = 289 mg/dl	High lipid risk.
	III	220.0	64.0	121.0	34.0	1.89	Low lipid risk.for atherogenesis.	Hig
9.	I	185.0	48.0	106.0	30.0	2.2	LDL/HDL ratio = 2.2	Total score = + 2
	II	216.0	56.0	133.0	32.0	2.66	Fasting STC = 185 mg/dl	High lipid risk .
	III	196.0	56.0	104.0	30.0	1.85	Low lipid risk for atherogenesis.	
10.	I	186.0	58.0	93.0	34.4	1.6	LDL/HDL ratio = 1.6	Total score = 0
	II	210.0	60.0	110.0	32.0	1.61	Fasting STC = 186 mg/dl	Indeterminate lipid risk
	III	206.0	66.0	106.0	33.4	1.6	Low lipid risk for atherogenesis.	for atherogenesis.
11.	I	182.0	42.0	116.0	23.6	2.76	LDL/HDL ratio = 2.76	Total score = +0.5
	II	210.0	50.0	133.0	26.4	2.66	Fasting STC = 182 mg/dl	Indeterminate lipid
	III	190.0	46.0	118.0	25.6	2.56	Low lipid risk case.	

1	2	3	4	5	6	7	8	9
12.	I	204.0	39.0	133.0	32.0	3.41	LDL/HDL ratio = 3.41	Total score = -2
	II	188.0	46.0	116.0	31.2	2.52	Fasting STC = 204 mg/dl	Low risk or individual is protected against
	III	210.0	48.0	129.0	32.4	2.68	Moderate risk for athero-genic complication.	atherogenic complications.
13.	I	156.0	36.0	82.0	37.2	2.27	LDL/HDL = 2.27	Total score = - 2.5
	II	168.0	48.0	88.0	40.0	1.66	Fasting STC = 156 mg/dl	Low lipid risk for athero genesis. Protected case
	III	160.0	40.0	88.0	38.0	2.05	Low lipid risk.	against atherogenic complications.
14.	I	238.0	38.0	164.0	35.2	4.31	LDL/HDL = 4.31	Total score = + 0.5
	II	266.0	43.0	183.0	39.2		Fasting STC = 238 mg/dl	Unpredictable lipid risk
	III	256.0	46.0	176.0	34.0		High lipid risk. Highly susceptible for athero-genic complications.	for atherogenesis.
15.	I	146.0	66.0	55.0	25.2	0.83	LDL/HDL ratio = 0.83	Total score = - 0.5
	II	158.0	74.0	55.0	28.2	0.74	Fasting STC = 146 mg/dl.	Indeterminate lipid risk
	III	148.0	70.0	50.0	27.2	0.71	Low risk.	for atherogenesis.
16.	I	166.0	48.0	94.0	23.6	1.95	LDL/HDL ratio = 1.95	Total score = + 2.5
	II	148.0	46.0	76.0	25.2	1.65	Fasting STC = 166 mg/dl	High risk for athero-
	III	170.0	42.0	106.0	22.0	2.52	Low lipid risk for atherogenic complications	genesis.
17.	I	226.0	48.0	144.0	34.0	3.0	LDL/HDL ratio = 3.0	Total score = +1
	II	202.0	40.0	130.0	32.0	3.25	Fasting STC = 226 mg/dl	High risk on feeding.
	III	216.0	44.0	138.0	33.6	3.13	Borderline lipid risk for atherogenesis.	

1	2	3	4	5	6	7	8	9
18.	I	156.0	45.0	85.0	25.6	1.88	LDL/HDL ratio = 1.88	Total score = +3.5. This Subject shows slightly unfavourable score on STF. Thus on STF, the lipid profile changes adversely - high risk.
	II	168.0	40.	101.0	26.8	2.52	Fasting STC = 156 mg/dl	
	III	160.0	40.0	92.0	27.6	2.3	Low lipid risk for atherogenesis.	
19.	I	163.0	38.0	97.0	27.6	2.55	HDL/HDL ratio = 2.55	Total score = +0.5
	II	176.0	46.0	100.0	29.6	2.17	Fasting STC = 163 mg/dl	Indeterminate lipid risk for atherogenic complication.
	III	195.0	36.0	130.0	28.5	3.61	Low lipid risk for atherogenesis.	
20.	I	210.0	98.0	120.0	31.2	2.06	LDL/HDL ratio = 2.06	Total score = -0.5
	II	188.0	96.0	103.0	20.8	1.83	Fasting STC = 210 mg/dl	Indeterminate lipid risk for atherogenic complications.
	III	190.0	94.0	106.0	29.6	1.96	Low lipid risk for atherogenic complication.	
21.	I	188.0	40.0	120.0	27.6	3.0	LDL/HDL ratio = 3.0	Total score = - 0.5
	II	156.0	46.0	84.0	26.0	1.82	Fasting STC = 188 mg/dl	Indeterminate lipid risk for atherogenesis.
	III	168.0	52.0	90.0	26.0	1.73	Low lipid risk for atherogenesis.	
22.	I	168.0	98.0	86.0	23.2	1.48	LDL/HDL ratio = 1.48	Total score = -3. Lipid lipoprotein profile changes occur favourable direction on STF - Protected state.
	II	140.0	63.0	55.0	24.4	0.88	Fasting STC = 168 mg/dl.	
	III.	150.0	60.0	66.0	24.0	1.10		
23.	I	32800	58.0	136.0	34.0	2.31	LDL/HDL ratio = 2.31	Total score = -3. A protected state on STF Very low atherogenic risk on STF.
	II	200.0	60.0	107.0	32.4	1.78	Fasting STC = 226 mg/dl.	
	III	216.0	52.0	136.0	31.2	2.5	Borderline or moderate lipid risk for atherogenesis.	
24.	I	225.0	56.0	133.0	36.0	2.37	LDL/HDL ratio = 2.37	Total score = -1.5
	II	238.0	60.0	139.0	38.4	2.31	Fasting STC = 225 mg/dl	Unpredictable lipid
	III	236.0	60.0	128.0	40.0	1.88	Moderate lipid risk.	risk on STF.

1	2	3	4	5	6	7	8	9
25.	I	180.0	60.0	88.0	31.6	1.46	LDL/HDL ratio = 1.46	Total score = -3.
	II	166.0	66.0	67.0	32.4	1.01	Fasting STC = 180 mg/dl	Protected state.
	III	160.0	60.0	60.0	32.0	1.13	Low risk.	
26.	I	236.0	36.0	164.0	35.2	4.55	LDL/HDL ratio = 4.55	Total score = +0.5.on STF
	II	250.0	40.0	172.0	38.0	4.30	Fasting STC = 236 mg/dl.	Indeterminate lipid risk
	III	256.0	40.0	170.0	37.2	4.45	High lipid risk(Prone	for atherogenesis
	IV	230.0	40.0	153.0	36.4	3.82	for developing athero-	Total score = 0 on prolonged
							sclerotic disorders)	feeding. Indeterminate
								scoring.
27.	I	230.0	68.0	115.0	42.0	1.69	LDL/HDL ratio = 1.69	Total score = 0(STF).
	II	210.0	76.0	92.0	39.2	1.21	Fasting STC = 230 mg/dl	Indeterminate lipid risk on
	III	246.0	76.0	130.0	41.6	1.68	(Borderline lipid risk	STF. Total score = +4(LTF)
	IV	240.0	56.0	144.0	40.0	2.57	for atherogenic	High risk on LTF.
							complications).	
28.	I	280.0	56.0	184.0	39.2	3.28	LDL/HDL ratio = 3.28	Total score = + 1.5
	II	276.0	48	192.0	35.6	4.0	Fasting STC = 280 mg/dl	High lipid risk for
	III	270.0	58.0	176.0	36.0	3.03	High lipid risk for	atherogenesis
							atherogenic complication.	
29.	I	200.0	56.0	112.0	31.2	2.0	LDL/HDL ratio = 2	Total risk = -2.5
	II	186.0	62.0	91.0	32.8	1.46	Low lipid risk for	Protected state.
	III	204.0	62.0	108.0	33.2	1.74	atherogenesis	
							Fasting STC = 200 mg/dl.	
30.	I	180.0	58.0	94.0	72.2	1.62	LDL/HDL ratio = 1.62	Total score = 0
	II	166.0	66.0	68.0	31.2	1.03	Fasting STC = 190 mg/dl	Indeterminate risk . Low
	III	188.0	60.0	100.0	28.0	1.66	Low lipid risk for	lipid risk for
							atherogenesis.	atherogenesis.
31.	I	218.0	50.0	135.0	32.4	2.7	LDL/HDL ratio = 2.7	Total score = -2
	II	206.0	64.0	102.0	33.6	1.59	Fasting STC = 218 mg/dl	Protected . Low lipid
	III	236.0	60.0	142.0	34.0	2.36	Low lipid risk for	risk for atherogenesis.
							atherogenesis.	

1	2	3	4	5	6	7	8	9
32.	I	190.0	48.0	106.0	35.6	2.20	LDL/HDL ratio = 2.20	Total score = +1
	II	206.0	46.0	117.0	36.8	2.54	Fasting STC = 190 mg/dl	Indeterminate lipid risk
	III	200.0	50.0	113.0	36.4	3.64	Low lipid risk for atherogenesis.	for atherogenesis.
33.	I	162.0	64.0	66.0	31.6	1.03	LDL/HDL ratio = 1.03	Total score = 0
	II	172.0	70.0	72.0	30.0	1.02	Fasting STC = 162 mg/dl	Unpredictable lipid risk
	III	170.0	68.0	72.0	30.8	1.05	Low lipid risk for atherogenesis.	for atherogenesis.
34.	I	194.0	46.0	115.0	32.6	2.5	LDL/HDL ratio = 2.5	Total score = + 2.5
	II	206.0	48.0	123.0	34.8	2.56	Fasting STC = 194.mg/dl	High risk on STF
	III	200.0	40.0	126.0	34.0	3.15	Less prone for atherogenic risk(Low lipid risk)	
35.	I	218.0	56.0	124.0	30.0	2.21	LDL/HDL ratio = 2.21	Total score = 0
	II	210.0	62.0	109.0	36.4	1.75	Fasting STC = 218 mg/dl	Unpredictable risk.
	III	216.0	50.0	130.0	36.0	2.60	Low lipid risk. Less prone for atherogenic risk.	
36.	I	144.0	56.0	64.0	24.0	1.14	LDL/HDL ratio = 1.14	Total risk = +0.5
	II	162.0	62.0	72.0	26.4	1.17	Fasting STC = 144 mg/dl	Unpredictable risk for atherogenesis.
	III	160.0	64.0	70.0	26.0	1.09	Low lipid risk. Less prone for atherogenesis.	
37.	I	176.0	46.0	100.0	30.0	2.17	LDL/HDL ratio = 2.7	Total score = +2
	II	182.0	52.0	96.0	33.8	1.84	Fasting STC = 176 mg/dl	High risk.
	III	200.0	50.0	119.0	30.8	2.38	Low lipid risk.	
38.	I	230.0	60.0	132.0	37.6	2.2	LDL/HDL ratio = 2.20	Total score = + 2.5
	II	246.0	58.0	152.0	35.6	2.62	Fasting STC = 230 mg/dl.	High lipid risk.
	III	226.0	50.0	142.0	34.0	3.84	Moderate lipid risk for atherogenic complications	

1	2	3	4	5	6	7	8	9
39.	I	210.0	50.0	130.0	36.0	2.48	LDL/HDL ratio = 2.48	Total score = +0.5
	II	228.0	54.0	136.0	37.2	2.51	Fasting STC = 210 mg/dl	Indeterminate lipid
	III	206.0	50.0	117.0	38.4	2.34	Low lipid risk.	risk.
40.	I	173.0	60.0	75.0	38.0	1.25	LDL/HDL ratio = 1.25	Total score = +4
	II	180.0	58.0	82.0	39.2	1.41	Fasting STC = 173 mg/dl.	High lipid risk on STF
	III	170.0	50.0	97.0	36.8	1.94	Low susceptibility for atherogenic complications.	
41.	I	182.0	60.0	84.0	38.0	1.4	LDL/HDL RATIO = 1.4	Total score = +3.5.
	II	210.0	68.0	100.0	41.2	1.47	Fasting STC = 182 mg/dl	High lipid risk. Possessing individual
	III	226.0	58.0	128.0	39.6	2.20	Low lipid risk.	on STF
42.	I	188.0	39.0	111.0	37.6	2.84	LDL/HDL ratio = 2.84	Total score = -4.5
	II	176.0	48.0	88.0	39.2	2.0	Fasting STC = 188 mg/dl.	Protected individual
	III	220.0	56.0	124.0	40.8	2.21	Low lipid risk case.	from atherogenic risk on STF.
43.	I	160.0	46.0	90.0	31.2	1.95	LDL/HDL ratio = 1.95	Total score = -4.5
	II	150.0	50.0	58.0	34.0	1.00	Fasting STC = 160 mg/dl	Protected from
	III	156.0	50.0	66.0	32.0	1.13	Low lipid risk for atherogenesis.	atherogenic risk.
44.	I	208.0	40.0	132.0	36.0	3.3	LDL/HDL ratio = 3.3	Total score = + 5.5
	II	230.0	33.0	165.0	39.2	5.0	Fasting STC = 208 mg/dl.	High lipid risk on STF
	III	218.0	36.0	144.0	38.0	4.0	Borderline lipid risk.	
45.	I	172.0	36.0	104.0	32.0	2.88	LDL/HDL ratio = 2.88	Total score = - 0.5
	II	206.0	38.0	122.0	35.2	2.56	Fasting STC = 172 mg/dl.	Indeterminate lipid
	III	218.0	58.0	133.0	34.4	2.66	Low lipid risk.	risk.

1	2	3	4	5	6	7	8	9
46.	I	216.0	60.0	125.0	30.4	2.08	LDL/HDL ratio = 2.08	Total score = +0.5
	II	238.0	72.0	132.0	33.6	1.83	Fasting STC = 216 mg/dl	Indeterminate lipid
	III	210.0	70.0	106.0	33.2	1.51	Low susceptibility for atherogenic complication.	risk.
47.	I	210.0	38.0	142.0	30.0	3.73	LDL/HDL ratio = 3.73	Total score = - 2.5
	II	236.0	56.0	152.0	27.5	2.71	Fasting STC = 210 mg/dl.	Protected individual.
	III	240.0	50.0	164.0	26.0	3.28	Borderline or moderate lipid risk for atherogenic complications.	
48.	I	202.0	60.0	114.0	28.0	1.90	LDL/HDL ratio = 1.9	Total score = -1.5
	II	226.0	64.0	130.4	31.6	2.03	Fasting STC = 202 mg/dl.	Indeterminate lipid
	III	230.0	56.0	142.0	31.2	2.56	Low lipid risk.	risk.

1. Considering the basal lipid profile level 3(6.25%) subjects had high risk, 8(16.66%) borderline risk and 37(77.08%) subjects had low risk for atherogenic complications.

2. Short term feeding (STF) seems useful in assessing individual risk on this arbitrary scale in present study. On the basis of this scale 15(31.2%) subjects were categorised as high risk subjects for STF while 11(22.9%) subjects showed a protected state. Remaining 22 subjects (45.7%) showed unpredictable risk on such feeding.

3. Long term feeding (LTF) was carried out in 2 subjects. Of these two, one showed an indeterminate risk after 15 days feeding (the same inference was drawn on STF), while other showed high risk on LTF (the STF in this case showed an unpredictable risk).

4. If criteria of protection is taken as - 4 or less than that the scale becomes less sensitive and yields the result as follows :

   2 (4.16%) subjects = High risk.
   2(4.16%) subjects = Protected state.
   44(91.6%) subjects = Indeterminate or unpredictable response.

DISCUSSION

DISCUSSION

Present study was taken to explore the possibilities of improving and refining the existing cholesterol tolerance test. Different dietary articles were used in search of an effective and practical cholesterol tolerance test.

I. EFFECT OF DIFFERENT AMOUNTS OF BUTTER ON LIPID LIPOPROTEIN PROFILE

The mean fasting STC value in 2 subgroups (protocol 1 and 2) were 189.1±24.1 mg/dl and 185.6±33.9 mg/dl respectively. This was within the normal limits as set my lipid research clinics. In protocol 1 STC started rising in most of the subjects and peaked at 2 hours. The difference from fasting levels was statistically significant. A similar response was seen on protocol 2, but here the rise at 2 hours was not statistically significant.

The two critical aspects of this rise were - a relatively early peak observed at just 2 hours and secondly the huge quantum of change in STC. The early peak can be explained by the presence of a liquid vehicle (milk) which facilitated rapid mobilisation of cholesterol fat diet from stomach to gut as the subjects were in fasting stage. Earlier workers (Collen et al, 1969; Stewart, 1954) also reported immediate post prandial

response in adults, in the form of rise at 2 hour, but the workers had used a large amount of cholesterol 5 g and 3.5 g of cholesterol respectively. What causes such a rapid absorption is not clear and other workers hav have also not explained this.

The huge quantum of rise in STC cannot be explained on the basis of existing literature. The rise in mean STC was 27 mg%. This huge rise is tantamout to more than 800 mg cholesterol in absolute terms(27 x 10 x 3 = 810 mg), an amount that is much more than the cholesterol eaten.

We propose that dietary cholesterol fat stimulus provokes mobilisation of cholesterol from different stores (subintimal pool, macrophages) and is responsible for transient huge rise in STC. Further studies on larger sample sizes are needed to confirm and explain this observation.

A relatively small quantum of rise in STC has been observed in protocol 2 when lesser amount of butter was given. This finding is consistant with the studies of other workers, who have shown that changes in serum cholesterol have linear relation with dietary cholesterol (Keys, 1984; Hegsted, 1986).

The subjects in protocol 1 showed a significant rise of LDL. The rise in LDL is consistent with

the studies of other workers (Applebaum-Bowden et al, 1984) who have shown that when large amount of dietary cholesterol fat load is given the STC increased with most of the increase occurring in LDL subfraction. HDL rise was not significant in this study. Changes in triglyceride levels were also insignificant despite a huge fat load. In protocol 2, no lipid lipoprotein parameters showed any significant change. Thus on the basis of our study it can be suggested that 100 g butter diet is an important leading dietary item to elicit significant changes in lipid lipoprotein profile and can be used as cholesterol fat load in cholesterol fat tolerance test. The problem in identifying abnormal response after such a load is there, we propose that quantum of rise in STC is proportional to degree of risk in an individual. On giving butter load the greater is the rise the more is the risk for atherogenic complications, because more cholesterol is released from large stores in such individuals. This hypothesis needs to be confirmed after studies on large segment of our population and more specifically by prospective studies.

II. EFFECT OF DIFFERENT AMOUNT OF EGGS ON LIPID LIPOPROTEIN PROFILE

Graded amounts of eggs (1 to 6) were given to healthy subjects in this study. When single egg was given to the 8 subjects, 75% of them showed a significant fall

at 1 hour. What causes this fall in STC is not very clear, but we think that LDL receptor mechanism is responsible for this. According to Joseph et al (1982), after an overnight fast, there occurs suppression of LDL receptors. We propose that when cholesterol fat load is given after an overnight fast these receptors are stimulated in anticipation of the cholesterol load that will enter the circulation.

So large amounts of LDL from the intravascular compartment shifts into the subendothelial pool resulting in an acute fall of LDL and STC at 1 hour. The cholesterol level slowly increase after 3 hours as a result of the absorbed cholesterol and the reverse movement of LDL that had entered the circulation earlier. The significant fall in LDL at first hour in this group of subjects, favours the working of this mechanism.

The rise of STC after feeding may be explained again on the basis of LDL receptor mechanism. In these subjects, these receptors are saturated probably because of large amount of cholesterol store in the vessel wall and elsewhere and are not stimulated by dietary cholesterol. Thus STC in these subjects increases at one hour because of absorbed dietary cholesterol. The STG and HDL were virtually unaffected in this group of persons. Using different protocols varying quantities of eggs (2,3,4 and 6) were given. STC showed a variable

response. Out of 8 subjects who were subjected to different amount of egg cholesterol 4 showed a rise at 1 hour while remaining 4 showed a fall. The rise and fall was within 5-8.5% of basal. The variability in response has also been observed by many other workers but on long term feeding (Flynn et al, 1979; Sacks, 1983). Why this variability of response occurs with egg cholesterol and not with butter cholesterol and other dietary cholesterol remains unanswered. This also poses the problem of identification of normal response (rise or fall) as all the subjects were healthy. Arora et al (1989) identified fall of STC and LDL at 1 hour as normal response as it occurred in majority of their subjects and offered above mechanism for such categorisation. In our series the pattern of rise and fall was seen in all most equal number of cases. The second important aspect of our study has been the observation that on increasing egg cholesterol dose, the magnitude of change is little affected. This is in accordance with the accepted fact that the amount of cholesterol absorbed is proportional to the amount eaten below the dietary cholesterol content of 500 mg. above this level dietary cholesterol has little effect on serum cholesterol (A report to the congress pursuant to the FSA of 1985, PL 99-198, S. 1453).

The other parameters in the study did not show consistent pattern of changes, however, in most of the subjects changes in LDL were parallel to changes in STC.

A loading dose of one (or at the most 2 eggs) would have been an effective stimulus for changes in lipid profile in the earlier proposed test.

III. EFFECT OF MISCELLANEOUS NON CHOLESTEROL DIETARY ITEMS ON SERUM LIPID LIPOPROTEIN PROFILE

There were little changes in different lipoprotein parameters with miscellaneous dietary items except when 'deshi ghee was used. In the latter case rise in STC, HDL, LDL and STG were seen. The deshi ghee contains very little cholesterol (60 mg/100 g) with basically fat rich in saturated fatty acids. Thus changes in lipoprotein profile at second postprandial hour by giving just 50 g of ghee are not explainable, specially the rise in serum total cholestero. We propose that dietary fats (or probably diet itself, as other non cholesterol food articles had also caused some change) evoke some neuronal or hormonal stimuli which set changes in lipid lipoprotein profile. Chenoweth (1982), Oh et al (1985) and Reggiani et al (1984) studied the effect of dietary fat on serum cholesterol and other lipoprotein fractions. The diet contained small amount of cholesterol. The feeding was conducted over a period of minimum 4 weeks. These workers also reported significant rise in serum cholesterol with little change in other lipid lipoprotein parameters. Little changes were observed when non cholesterol fatty diets or even

cholesterol fat free diet (Glucose) were used. This may be because of our method being less sensitive (Precipitation method) or stimulation of diet as such bringing about these changes. Carell et al (1983) also showed changes in lipid lipoprotein profile with other dietary items.

EFFECT OF CRYSTALLINE CHOLESTEROL ON SERUM LIPID LIPOPROTEIN PROFILE

When 1 g crystalline cholesterol was given to 8 subjects there was small but significant rise in serum cholesterol in most of the subjects. This has also been shown by some other workers on long term basis (Conner, et al (1961) showed that crystalline cholesterol is slowly and incompletely absorbed. This may be partially responsible for small rise in STC (incomparison to butter cholesterol induced changes). In this study, while some subjects showed a rise of 725 mg/dl other showed a smaller rise or even slight fall. The variability in response on cholesterol feeding we has well been documented by several workers (Oh and Miller,1985; Katan et al, 1986). The latter worker classified subjects on basis of their response to cholesterol fat load as hyper and hypo-responders. The rise in STC was mainly because of rise in LDL. Triglyceride level also showed significant rise, which is not explainable and needs to be confirmed by further trials.

EFFECT OF PROLONGED FEEDING

Two subjects were given a breakfast consisting of 2 eggs and 200 ml of milk for 15 days. This diet providing about 600 mg of cholesterol was not being taken by them previously. STC and other lipoprotein parameters showed little (insignificant) change after 15 days of feeding. This finding was consistent with the work of Glynn et al (1979), who showed that modification of dietary cholesterol by including or excluding eggs in diet does not significant alter STC level even after feeding for 6 weeks. Some other workers (Chenoweth et al, 1982, Schvenfeld et al, 1982) showed significant increment in STC and LDL level after prolonged feeding, but cholesterol amount, they used were huge (1000 mg/day).

REPRODUCIBILITY OF FEEDING BEHAVIOUR

We assessed reproducibility of responses in two of our subjects after a gap of 15 days and were able to show 'preserved qualitative changes' one of our subject who showed fall in STC and LDL at one hour after receiving high fat cholesterol diet duplicated the behaviour after 15 days, though the absolute values were considerably different on latter occasion. In the second subject, when the response was retested it was found to be reproducible.

Reproducibility of response after short or long

term feeding has always been a debatable point. Beynen and Katan (1985) showed the reproducibility of response after 3 years, while some other workers (Dawber et al, 1982) were unable to reproduce the responses. Probably further studies on large number of volunteers with more advanced method of lipoprotein estimation should be employed to have a definite answer.

CHOLESTEROL FAT TOLERANCE TEST : NEED FOR IMPROVEMENT AND REFINEMENT

The concept of cholesterol fat tolerance test is a very complex one. The present study has definitely pointed towards some of the flaws in existing test devised by Arora et al (1989).

1. In the present study the fall of STC and LDL at first postprandial hour after feeding of HCFB is seen in about half of the normal healthy volunteers who received egg diet. So labelling this response as normal and other responses (rise or no change) as abnormal is not justified. This variability in response is a normal phenomena, pointed out by various other workers (vide supra),. Immediate effect of HCFB (i.e. single point HCFB feeding, as a determinate of individual risk does not seem justified because of variations among individuals in responsiveness to dietary cholesterol.

2. In the present study when 2 individuals who were earlier subjected to a single dose HCFB when

later given a prolonged (15 days) feeding showed virtually no effect in lipoprotein parameters. This further strengthens the view that single point feeding induced responses are normal variation rather than specific risky or non risky responses.

3. Subjects who were given butter and crystalline cholesterol showed consistent rise rather than variability in responses. This suggests that there is some substance in egg yolk which is responsible for this variability.

4. Non cholesterol dietary items also elicit some although little changes in STC and other lipoprotein parameters. This fact further questions the validity of such a test.

On the basis of our study we recommend following changes in a prospective cholesterol fat tolerance test.

1. As large segment of our population consists of vegetarians. Further study is needed with butter cholesterol and crystalline cholesterol. Our hypothesis that degree of rise in STC and LDL is proportional to risk of atherogenesis needs to be verified by prospective studies.
2. The study with egg cholesterol should have more wide coverage. Graded amount of eggs should be given to same individual and the responses each

time should be compared. The individual who have showed a consistent pattern of response on butter cholesterol should be given egg cholesterol and vice versa. The subjects who are presumed to be at higher risk by convention cholesterol tolerance test (i.e. rise of STC and LDL at one hour) should undergo a prolonged feeding for example 2 weeks and the changes in them should show an unfavourable lipid lipoprotein profile after this feeding.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present work was carried out in 31 male and 17 female healthy volunteers of 15-60 years of age. They were subjected to different types of dietary loads (single dose) in search of an effective, practically feasible and simple cholesterol tolerance test.

Ten subjects were given a test diet consisting of 100 g butter based diet. Postprandial significant rise in levels of STC, HDL and LDL were observed in 90% of the subjects at 2nd hour. STG level did not show significant change. Values returned to near basal level at 5th postprandial hour. Five subjects who were given a test diet based on 50 g butter, showed rise in all parameters but the changes were insignificant.

In an another protocol different amount of egg yolk used as dietary test load. In eight subjects who were given a single hen egg (boiled) STC, and LDL fall at first postprandial hour in 75% of subjects. This fall was statistically significant. Other parameters showed insignificant changes. In 25% of cases all parameters increased insignificantly when increasing amount of egg cholesterol (from 2-6 eggs) were given to another eight subjects, there was marked variability in the response, while half of them showed rise of STC and LDL at first postprandial hour, remaining half showed fall. The changes were statistically not significant.

Eight subjects were fed miscellaneous dietary articles viz. egg albumin, 75 g of glucose, 50 g ghee, 50 g saffola oil and 50 ml of alcohol. The changes in lipid lipoprotein profile were less marked except in case of pure ghee where STC and STG showed great quantum of increase. Other dietary articles also showed changes in the form of rise, though the quantum of rise was not great.

In a fourth group of subjects, crystalline cholesterol (1 g) dissolved in 200 ml of milk was given. In most of the subjects (75%) there was rise in STC, STG and HDL (statistically significant) while HDL was insignificant increased, 25% of cases showed fall at first hour postprandially.

Two subjects were subjected to prolonged feeding (2 weeks). The changes in lipid lipoprotein profile were not significant. Reproducibility of test was also assessed in these subjects. The feeding behaviour was found to be reproducible qualitatively.

The following conclusions were drawn from the present study.

1. 100 g butter based diet produced significant changes in lipid lipoprotein profile in most of the subjects. This change was in the form of rise.
2. The rise was more marked in subjects having comparatively low STC level.

3. The changes in lipid lipoprotein profile were less marked and insignificant when 50 g butter diet was given.

4. The egg cholesterol induced changes were found to be highly variable contrast to butter cholesterol. Single egg feeding produced fall in STC and LDL in majority of subjects while increasing egg cholesterol disturbed this consistency and produced variability of the responses.

5. Changes in lipid lipoprotein profile were little influenced by amount of egg yolk cholesterol in test diet i.e. on increasing egg yolk cholesterol (2 to 6), changes in lipid lipoprotein profile showed same quantum of changes as with one egg.

6. Subjects who had comperatively higher basal STC showed little change after feeding while those with normal STC level showed more marked changes (rise or fall) after feeding.

7. Subjects in whom STC was destined to fall at one hour, it started falling from very begining.same was true for rise of STC after feeding test diet.

8. The egg yolk induced changes in lipid lipoprotein profile were not very marked excluding few individual cases.

9. Non cholesterol fatty articles - saffola oil and egg albumin elicited very little variable changes

in lipid profile. In the same way cholesterol fat free articles alcohol, glucose etc. elicited similar little variable postprandial changes.

10. Changes induced by ghee were more marked with greater quantum of changes in STC, HDL and STG.

11. Crystalline cholesterol induced significant changes in lipid lipoprotein profile. Most of the subjects showed rise. The rise in different parameters was variable while some individuals responding with greater quantum of changes, other showed minor changes.

12. Prolonged feeding with 2 eggs and 250 ml of milk induced no change in lipid lipoprotein profile.

13. Reproducibility of feeding behaviour was well elicited in 2 of the subjects after 15 days of initial test.

14. Individual dietary risk assessment on the basis of an arbitrary scale showed an indeterminate and unpredictable risk in about half of the cases. This unpredictability of risk assessment was increased when egg yolk and non cholesterol fatty articles are given.

B I B L I O G R A P H Y

BIBLIOGRAPHY

1. Abraham S, Carroll MD : Fats,cholesterol and sodium intake in the diet of persons 1-74 years : United States. Advance data vital Health State, 54 :1-9;1981.
2. Applebaum Bowden D, Haffner SM, Hartsook E, Lak KH, Albers JJ, Hazzard WR : Down regulation of low density lipoprotein receptor by dietary cholestero. Am J Clin Nutr, 39 : 360-367; 1984.
3. Albrink MJ, Man EB : Effect of carbohydrate ingestion on post prandial lipemia. Clin Res Proc, 4:121; 1956.
4. Arora RC, Agarwal N, Arora S, Garg RK, Gupta G : High fat and cholesterol diet induced changes in plasma cholesterol and lipoprotein in healthy human volunteers. J.A.P.I. 35 : 774-775; 1987.
5. Arora RC, Agarwal N, Arora S, Mehra V, Garg RK : Triglyceride tolerance test : is it feasible. Materia Medica Polona, 19 : 85-89; 1987.
6. Arora RC, Agarwal, N, Arora S, Mangal R : The evaluation of the changes in lipid lipoprotein profile induced after ingestion of single high cholesterol test diet. Thesis for M.D.(Medicine), 1989.
7. Barrit DW : Alimentary lipemia in men with coronary artery disease and in controls. Brit Med J, 2 : 640; 1956.

8. Baudet MF, D Esteve, Delplanque B, Minchenne M and Jacotot B : Effect of three dietary fats on plasma lipids and lipoproteins in fasting and postprandial human after a short term diet. Lipids 15(4) : 216-223; 1981.

9. Beynen AC, Katan MB : Reproducibility of the variations between humans in the response of serum cholesterol to cessation of egg consumption. Atherosclerosis. 57 : 19-21; 1985.

10. Beynen AC, Katan MB : Inter-individual variation in the cholesterolemic response to dietary cholesterol. Prog Clin Biol Res, 188 : 195-207; 1985 C.

11. Bunyard IM, Mc Roberts MR, Driscoll DL, Bowering J : Effect of dietary eggs and ascorbic acid on plasma lipid and lipoprotein cholesterol levels in healthy young men. Am J Clin Nutr., 36 : 94-105; 1982.

12. Carroll KK : Hypercholesterolemia and atherosclerosis : Effects of dietary protein. Fed. Proc. 41 : 2792; 1982.

13. Chenoweth W, Ullmann M, Simpson R, Leveille G : Influence of dietary cholesterol and fat on serum lipids in man. J Nutr, 111 : 2069-2080; 1981.

14. Carlson LA, and Ericsson M : Quantitative and qualitative serum lipoprotein analysis in healthy men and women. Atherosclerosis, 21 : 417-433;1975.

15. Castelli WP, Doyle JT, Gordon T et al : Alcohol and blood lipids. The co-operative lipoprotein phenotyping study. Lancet, 2 : 153-155; 1977.

16. Connors WE, Hodge RE and Sleiler RE : Serum lipids in men receiving high cholesterol and cholesterol free diet. J Clin Invest, 40 : 894; 1961.

17. Dawber TR, Nickerson RJ, Brand FN, Pool J : Eggs, serum cholesterol and coronary heart disease. Am J Clin Nutr, 36 : 617-625; 1982.

18. Fredrickson DS, Goldstein JL and Brown MS : The familial hyperlipoproteinemias : The metabolic basis, 1978.

19. Fredrickson DS : A physician guide to hyperlipidemia. Med Concepts Cardiovasc Dis, 41 : 31; 1972.

20. Flynn MA, Nolph GB, Flynn TC, Kahrs R, Krause G : Effect of dietary egg on human serum cholesterol and triglycerides. Am J Clin Nutr, 32 : 1051-1057;1979.

21. Ginsberg H, Le N-A, Mays C, Gibson J, Brown WV : lipoprotein metabolism in non responders to increased dietary cholesterol. Arteriosclerosis,1:463-70;1981.

22. Goldstein JL and Brown MS : Atherosclerosis : The low density lipoprotein receptor hypothesis. Metabolism, 26 : 1257-1275; 1977.

23. Goldstein JL, Brown MS : Binding and degradation of low density lipoprotein by cultured human fibroblasts. J Biol Chem, 249 : 5153; 1974.

24. Gordon T, Castelli WP, Hjorland MC et al : High density lipoprotein as a protective factor against coronary heart disease. The Framingham study. Am J Med, 62 : 707; 1977.

25. Gordon T, Kannel WB, Castelli WB, Dawber TR : The lipoproteins, cardiovascular disease and death. The Framingham study. Arch Intern Med, 141 : 1128;1981.

26. Menno Krauss, Dieter Groot, Buen van Ram Sherst et al: Chylomicron metabolism in coronary atherosclerosis. Circulation Supp. Part II, 76(4) : 1987.

27. Hegsted DM : Serum cholesterol response to dietary cholesterol : a re-evaluation. Am J Clin Nutr, 44 : 299-305; 1986.

28. Heiss G, Tamir I, Davis CE et al : Lipoprotein cholesterol distributions in selected North American populations. The lipid research clinics program prevalence study. Circulation, 61 : 302-315; 1962.

29. James F, Sullivan : The effect of protein ingestion on alimentary lipemia. Am J Med Sci, 243:770; 1962.

30. Jacobs DR Jr, Andersen JT, Hannan P, Keys A, Blackburn H : Variability in individual serum cholesterol response to change in diet. Arteriosclerosis, 3 : 349-356; 1983.

31. Kannel WB, Castelli WP, Gordon T : Cholesterol in the prediction of atherosclerotic disease. Ann Intern Med, 90 : 85; 1979.

32. Kay RM, Rao S, Arnott C et al : Acute effects of the pattern of fat ingestion on plasma HDL components in man. Atherosclerosis, 36 : 567-573; 1980.

33. Katan MB, Beynen AC, Devries JHM, Nobels A : Existence of consistent hypo and hyperresponders to dietary cholesterol in man. Am J Epidemiol, 123:221-234;1986.

34. Keys A : Serum cholesterol response to dietary cholesterol. Am J Clin Nutr, 40 : 351-359; 1984.

35. Keys A : Coronary heart disease, serum cholesterol, and the diet. Acta Med Scand, 207 : 153-160;1980.

36. Kmuiman JT, West CE : The concentration of cholesterol in serum and in various serum lipoproteins in macrobiotic vegetarian and non vegetarian men and boys. Atherosclerosis, 43 : 71; 1982.

37. Kritchevsky D and Tepper SA : Influence of medium chain triglycerides on cholesterol metabolism in rats. J Nutr, 96 : 67-72; 1968.

38. Liebman M, Bazzarre TL : Plasma lipids of vegetarian and non vegetarian males : Effects of egg consumption. Am J Clin Nutr, 38 : 612-619; 1983.

39. Mahley RW, Innerarity TL, Barset TP, Lipson A ; Margolis S : Alterations in human high density lipoproteins, with or without increased plasma cholesterol induced by diets high in cholesterol. Lancet, 2 : 867-869; 1978.

40. Mahley RW, Dietary fat, cholesterol and accelerated atherosclerosis. Atherosclerosis Review, New York,

41. Mahley RW : The role of dietary fat and cholesterol in atherosclerosis and lipoprotein metabolism.

42. Mahley RW and Holcombe KS : Alterations of the plasma lipoprotein and apoprotein following cholesterol feeding in rats. J Lipids Res,18;314-24;1977.

43. Mc Namara DJ : Diet and hyperlipidemia : A justifiable debate. Arch Intern Med, 42 : 1121-1124; 1982.

44. Mistry P, Miller NE, Laker M, Hazzard WR, Lewis B : Individual variation in the effects of dietary cholesterol on plasma lipoprotein and cellular cholesterol homeostasis in man. studies of low density lipoprotein receptor activity and 3-hydroxy 3 methyl glutaryl co-enzyme a reductase activity in blood mononuclear cells. J Clin Invest, 67 : 493-502; 1981.

45. Nikkila EA, Konttinen A : Effect of physical activity on postprandial levels of fat in serum. Lancet, 1151; 1962.

46. Nestel PJ : Fish oil attenuates the cholesterol induced rise in lipoprotein cholesterol. Am J Clin Nutr, 43 : 752-757; 1986.

47. Oh SY, Miller LT : Effect of dietary egg on variability of plasma cholesterol levels and lipoprotein cholesterol. Am J Clin Nutr, 42 : 421-431; 1985.

48. Oliver MF : Diet and coronary heart disease. Human Nutr. Clin Nutr, 366 : 413-427; 1982.

49. Reiser S, Hellpish J, Michadis OP et al : Isocaloric exchange of dietary starch and sucrose in human. Am J Clin Nutr, 32 : 1659; 1978.

50. Sachs FM, Miller L, Sutherland M, Albers JJ, Salazar J, Foster JM, Samonds KW, Kass EH : Ingestion of egg raises plasma low density lipoproteins in free living subjects. Lancet, 647-649; 1984.

51. Shepherd J, Packard CJ, Bicker S, Lawrie TDV, Morgan HG : N Engl J Med, 302 : 1219-1222; 1980.

52. Slack and Evans : The increased risk of death from ischaemic heart disease in first degree relatives of 121 men and 96 women with ischaemic heart disease.

53. Samuel P, Mc Namara DJ, Shapiro J : The role of diet in the etiology and treatment of atherosclerosis. Ann Rev Med, 34 : 179-196; 1983.

54. Shekelle RB, Shryock AM, Paul O, Lepper M, Stamler J, Liu S, Raynor WJ : Diet, serum cholesterol and death from coronary heart disease. The western Electric study. N Eng J Med, 304 : 65-70; 1981.

55. Simons LA, Gibson JC, Paino C, Hosking M, Bullock J, Trim J : The influence of a wide range of absorbed cholesterol on plasma cholesterol levels in man. Am J Clin Nutr, 31 : 1334-1339; 1978.

56. Sinclair H. Dietary fats and coronary heart disease. Lancet, 1 : 414-415; 1980.

57. Stallones RA : Ischemic heart disease and lipids in blood and diet. Annu Rev Nutr.3 : 155-185;1983.

58. Stein EA, Shapero J, Mc Nerney C, Glueck CJ, Tracy T, Gartside P : Changes in plasma lipid and lipoprotein fractions after alteration in dietary cholesterol, polyunsaturated, saturated and total fat in free living normal and hypercholesterolemic children. Am J Clin Nutr, 35 : 1378-1390; 1982.

59. St Clair RW : Atherosclerosis regression in animal models, current concepts of cellular and biochemical mechanism. Prog Cardiovas. Dis, 26 : 109; 1983.

60. Tan Bardan, Wissler RW, Hughes RH : A physiochemical study of the lipoprotein system of the normal and estrogen treated male rats in relation to atherosclerosis. J Atherosclerosis Res, 4 : 477-496;1964.

61. Tan MH ; Dickinson MA, Albers JJ et al : The effect of high cholesterol and saturated fat diet on serum high density lipoprotein cholesterol apoprotein A-I and apoprotein levels in normolipidemic humans. Am J Clin Nutr, 33 : 255; 1980.

62. Thompson PD, Jaffery RW, Wing RR et al : Unexpected decrease in plasma high density lipoprotein with weight loss. Am J Clin Nutr, 32 : 2016-2021; 1979.

63. Thomas CB : Familial patterns in hypertension and coronary heart disease. Circulation, 20 : 25;1959.

64. Vergnaic C and Bottalo A : Familial hypoalpha lipoproteinemia. Clin Chem Acta, 114:45-52; 1981.

65. Weidman WH, Ziveback LR, Nelson RA et al : Nutrient intake and serum cholesterol levels in normal children 6 to 16 years of age. Pediatrics, 61:354;1978.

66. Widholm K, Maxa E and Zyman I : Effect of diet and exercise upon the cholesterol and cholesterol content of plasma lipoproteins in overweight children. Eur J Paediatr, 127 : 121-126; 1978.

67. Wilson PE and Lees RS : Metabolic relationship among the plasma lipoproteins. Reciprocal changes in the concentration of very low and low density lipoprotein in man. J Clin Invest, 51 : 1051-1057; 1972.

68. Wood PD, Haskell WL, Stern MP et al : Plasma lipoprotein distribution in male and female runner. Ann N.Y. Acad Sci, 301 : 748-763; 1977.

69. Walker ARP : Dietary fat intake and serum cholesterol levels in coronary heart disease. S Afr Med J, 58 : 7-12; 1980.

70. White EC, Mc Namara DJ, Ahrens EH Jr : Validatum of dietary record system for the estimation of daily cholesterol intake in individual outpatients. Am J Clin Nutr, 34 : 199-203; 1981.

71. Wyenbenga DR et al : Clin Chem, 16 : 980; 1970.

72. Yano K, Reed DM, Curb JD, Rankin JM, Albers JJ : Biological and dietary correlates of plasma lipids and lipoproteins among elderly Japanese men in Hawaii. Arteriosclerosis, 6 : 422-433; 1986.

73 : Zilversmit DB : A proposal linking atherogenesis to the interaction of endothelial lipoprotein lipase with triglyceride rich lipoproteins. Cove Res, 33 : 633-638; 1973.

MASTER CHART

Sl. No.	Name of subject	Group/ Protocol	Age (Years)	Weight (kg)	Height (cm)	Serum Total Cholesterol I	II	III	IV	V
1.	R Kumar	A/1	21	65	166	207	220	237	227	220	222
2.	AK Gupta		28	74	162	225	247	256	218	202	212
3.	Vimal Agha		22	62	157	219	230	212	-	-	236
4.	Bipin Goel		22	63	160	162	180	186	170	-	165
5.	V Agarwal		22	68	162	218	200	212	-	-	210
6.	S Lakhatakia		26	72	164	176	298	200	187	180	182
7.	Km Anita		15	43	152	178	206	210	194	-	200
8.	Govind Bati		30	47	154	209	222	240	-	-	220
9.	Savitri		35	58	152	185	207	216	196	-	190
10.	Mamai		22	45	148	186	208	210	200	208	206
11.	DN Sharma	A/2	46	118	164	182	196	210	190	186	190
12.	Phool Kali		40	46	152	204	186	188	196	-	210
13.	PK Gupta		26	56	162	156	162	168	-	-	160
14.	PC Jain		40	66	156	238	262	266	-	-	256
15.	S Chandra		22	58	160	146	140	158	150	148	148
16.	VK Gupta	B/3	20	48	159	166	148	—	170	-	-
17.	S. Lal		34	58	162	226	202	-	216	-	-
18.	S Kumar		27	58	158	156	168	-	160	-	-
19.	Manik Lal		32	66	160	163	176	-	195	-	-
20.	Raisa Begum		35	56	154	210	188	-	190	-	-
21.	Guddo		23	48	148	188	156	-	168	-	-
22.	Marwada		18	46	148	168	140	-	150	-	-
23.	Urmila		28	48	150	226	200	-	218	-	-
24.	Malkhan Singh	C/4	42	68	164	225	238	-	236	-	-

25.	Maya Devi	C/4	28	52	154	180	166	-	160	-	-
26.	Shashi Kala	C/5	32	50	156	236	250	-	256	-	-
27.	AR Gupta		35	56	157	230	210	-	246	-	-
28.	DS Rawat	C/6	39	59	157	280	276	-	270	-	-
29.	Sahdev Singh		26	50	156	200	186	-	204	-	-
30.	Manu Tondon	C/7	18	68	157	180	166	-	188	-	-
31.	Gayadeen		26	58	160	218	200	-	236	-	-
32.	Lalanju	D/8	60	66	156	190	200	206	200	-	-
33.	Malti		26	51	156	162	172	170	170	-	-
34.	Meeradevi	D/9	28	48	154	194	206	200	200	-	-
35.	Radhey Shyam		55	70	160	218	210	220	216	-	-
36.	Ganesh	D/10	15	40	152	144	162	168	160	-	-
37.	Mannalal		26	47	162	176	182	196	200	-	-
38.	Mod.Zameel	D/11	30	62	164	230	246	238	226	-	-
39.	R Kumar		26	54	162	210	228	216	206	-	-
40.	Mohan Lal	D/12	28	60	160	173	180	186	170	-	-
41.	M Singh	E/13	29	70	164	182	210	218	226	-	-
42.	B Prasad		35	52	156	188	176	-	210	-	-
43.	Babulal		28	63	164	168	150	-	156	-	-
44.	Ramesh		20	54	160	208	238	226	218	-	-
45.	Tijia Bai		32	51	148	172	206	200	218	-	-
46.	Man Kunwar		35	48	150	216	238	216	210	-	-
47.	Bitola Devi		32	49	152	210	236	238	240	-	-
48.	Shimbu Bai		28	45	148	202	226	-	230	-	-

Protocol 14 : Subjects of protocol 5 were again studied after 15 days to assess long term effect of feeding & reproducibility of cholesterol tolerance test.

Sl. No.	Serum HDL (mg/dl)						Serum LDL (mg/dl)					
	I	X	II	III	IV	V	I	X	II	III	IV	V
1.	56	72	76	60	52	55	118.2	114.0	123.4	133.8	134.0	133.4
2.	52	56	60	66	50	50	141.8	159.0	162.4	120.0	120.4	131.6
3.	56	58	60	-	-	56	123.8	134.4	114.0	-	-	141.5
4.	38	35	48	40	-	35	98.0	116.0	108.0	100.8	—	102.00
5.	56	45	47	-	-	50	128.8	119.4	130.6	-	-	120.0
6.	45	58	56	55	50	54	103.0	110.4	113.2	102.0	100.8	98.0
7.	44	56	60	40	-	60	108.0	122.8	122.0	126.5	-	113.2
8.	58	60	66	-	-	64	117.0	125.2	138.0	-	-	121.2
9.	48	56	50	44	-	56	106.2	119.4	133.6	122.0	-	104.00
10.	58	64	68	60	62	66	93.6	115.6	110.0	107.2	114.0	106.6
11.	42	50	50	56	50	46	116.4	120.8	133.6	108.0	109.2	118.4
12.	39	44	46	40	-	48	133.0	111.6	116.8	124.4	-	129.6
13.	36	44	48	-	-	40	82.8	78.8	80.0	-	-	82.0
14.	38	40	43	-	-	46	164.8	184.0	183.8	-	-	176.0
15.	66	70	74	70	62	70	54.8	42.4	55.6	54.0	60.0	50.8
16.	48	46	-	42	-	-	94.4	76.8	-	106.0	-	-
17.	48	40	-	44	-	-	144.0	130.0	-	138.4	-	-
18.	45	40	-	40	-	-	85.4	101.2	-	892.4	-	-
19.	38	46	-	36	-	-	97.4	100.4	-	130.5	-	-
20.	58	56	-	54	-	-	120.8	103.2	-	106.4	-	-
21.	40	46	-	52	-	-	120.4	84.0	-	90.0	-	-
22.	58	62	-	60	-	-	86.8	55.6	-	66.0	-	-
23.	58	60	-	52	-	-	134.0	107.6	-	134.8	-	-
24.	56	60	-	68	-	-	133.0	139.6	-	128.0	-	-

25.	60	66	-	60	-	-	38.4	67.6	-	68.0	-	-
26.	36	40	-	40	-	-	164.8	172.0	-	178.8	-	-
27.	68	76	-	76	-	-	115.2	92.4	-	128.4	-	-
28.	56	48	-	58	-	-	184.8	192.4	-	176.0	-	-
29.	56	62	-	62	-	-	112.8	91.2	-	108.8	-	-
30.	58	66	-	60	-	-	94.8	68.8	-	100.0	-	-
31.	50	64		60	-	-	135.6	102.4	-	142.0	-	-
32.	48	46	56	50	-	-	106.4	117.2	114.8	113.6	-	-
33.	64	70	66	68	-	-	66.4	72.0	73.2	72.0	-	-
34.	46	48	52	40	-	-	115.4	123.2	114.0	126.0	-	-
35.	56	62	50	54	-	-	124.0	109.6	130.8	130.0	-	-
36.	56	62	60	64	-	-	64.0	73.6	82.8	70.0	-	-
37.	46	52	54	50	-	-	100.0	96.4	110.0	119.2	-	-
38.	60	58	56	50	-	-	132.4	152.4	147.6	142.0	-	-
39.	50	54	50	50	-	-	124.0	136.8	128.8	117.6	-	-
40.	60	58	50	50	-	-	75.0	82.8	97.6	83.2	-	-
41.	60	68	72	58	-	-	84.0	100.8	116.0	128.4	-	-
42.	39.	48	-	56	-	-	111.4	88.8	-	124.0	-	-
43.	46	58	-	58	-	-	90.8	58.0	-	66.0.	-	-
44.	40	33	30	36	-	-	132.0	165.8	159.6	144.0	-	-
45.	36	48	42	50	-	-	104.0	122.8	122.4	133.2	-	-
46.	60	72	66	70	-	-	125.6	132.4	115.5	106.8	-	-
47.	38	56	50	50	-	-	142.0	152.8	149.6	164.0	-	-
48.	60	64	-	56	-	-	114.0	130.4	-	142.8	-	-

Sl. No.	Serum Triglyceride (mg/dl)						Serum VLDL (mg/dl)					
	F	I	II	III	IV	V	F	I	II	III	IV	V
1.	164	170	180	166	170	168	32.8	34.0	37.6	33.2	34.0	33.6
2.	156	160	160	160	158	152	31.2	32.0	33.6	32.0	31.6	30.4
3.	196	188	190	-	-	192	39.2	37.6	38.0	-	-	38.5
4.	130	142	150	146	-	140	26.0	28.4	30.0	29.2	-	28.0
5.	166	178	172	-	-	170	33.2	35.6	34.4	-	-	34.0
6.	140	148	154	150	146	150	28.0	29.6	30.8	30.0	29.2	30.0
7.	130	136	140	136	-	134	26.0	27.2	28.0	27.5	-	26.8
8.	170	184	180	-	-	174	34.0	36.8	36.0	-	-	34.8
9.	154	158	162	150	-	150	30.8	31.6	32.4	30.0	-	30.0
10.	172	168	160	164	160	168	34.4	33.6	32.0	32.8	32.0	33.4
11.	118	126	132	130	134	128	23.6	25.2	26.4	26.0	26.8	25.6
12.	160	152	156	158	-	162	32.0	30.4	31.2	31.6	-	32.4
13.	186	196	200	-	-	190	37.2	39.2	40.0	-	-	38.0
14.	176	190	196	-	-	170	35.2	38.0	39.2	-	-	34.0
15.	126	138	142	130	130	136	25.2	27.6	28.4	26.0	26.0	27.2
16.	118	126	-	110	-	-	23.6	25.2	-	22.0	-	-
17.	170	160	-	168	-	-	34.0	32.0	-	33.6	-	-
18.	128	134	-	138	-	-	25.6	26.8	-	27.6	-	-
19.	138	148	-	142	-	-	27.6	29.6	-	28.5	-	-
20.	156	144	-	148	-	-	31.2	28.8	-	29.6	-	-
21.	138	130	-	130	-	-	27.6	26.0	-	26.0	-	-
22.	116	122	-	120	-	-	23.2	24.4	-	24.0	-	-
23.	170	162	-	156	-	-	34.0	32.4	-	31.2	-	-
24.	180	192	-	200	-	-	36.0	38.4	-	40.0	-	-

25.	158	162	-	160	-	-	31.6	32.4	-	32.0	-	-
26.	176	180	-	186	-	-	35.2	38.0	-	37.2	-	-
27.	210	196	-	208	-	-	42.0	39.2	-	41.6	-	-
28.	196	178	-	180	-	-	39.2	35.6	-	36.0	-	-
29.	156	164	-	166	-	-	31.2	32.8	-	33.2	-	-
30.	136	156	-	140	-	-	27.2	31.2	-	28.0	-	-
31.	162	168	-	170	-	-	32.4	33.6	-	34.0	-	-
32.	178	184	176	182	-	-	35.6	36.8	35.2	36.4	-	-
33.	158	150	154	150	-	-	31.6	30.0	30.8	30.0	-	-
34.	163	174	170	170	-	-	32.6	34.8	34.0	34.0	-	-
35.	190	182	176	180	-	-	38.0	36.4	35.2	36.0	-	-
36.	120	132	126	130	-	-	24.0	26.4	25.2	26.0	-	-
37.	150	168	160	154	-	-	30.0	33.6	32.0	30.8	-	-
38.	188	178	172	170	-	-	37.6	35.6	34.4	34.0	-	-
39.	180	190	186	192	-	-	36.0	38.0	37.2	38.4	-	-
40.	190	196	192	184	-	-	38.0	39.2	38.4	36.8	-	-
41.	190	206	200	198	-	-	38.0	41.2	40.0	39.6	-	-
42.	188	196	-	200	-	-	37.6	39.2	-	40.0	-	-
43.	156	170	-	160	-	-	31.2	34.0	-	32.0	-	-
44.	180	196	182	190	-	-	36.0	39.2	36.4	38.0	-	-
45.	160	176	178	172	-	-	32.0	35.2	35.6	34.4	-	-
46.	152	168	172	166	-	-	30.4	33.6	34.5	33.2	-	-
47.	150	136	142	130	-	-	30.0	27.5	28.4	26.0	-	-
48.	140	158	-	156	-	-	28.0	31.6	-	31.2	-	-

Sl. No.	Short Interval Study						Prolonged feeding study		
	F	A	B	C	I	III	F	I	III
				Serum Total Cholesterol (mg/dl)					
26.	236	240	246	246	250	256	230	242	240
27	230	218	206	220	210	246	240	226	230
				Serum High Density Lipoprotein (mg/dl)					
26.	36	30	30	38	40	40	40	48	54
27.	68	74	72	74	76	76	56	64	60
				Serum Triglyceride (mg/dl)					
26.	176	180	186	186	190	186	182	196	180
27.	210	206	200	204	196	208	200	178	186
				Serum Low Density Lipoprotein (mg/dl)					
26.	164.8	174.0	178.8	170.8	172.0	178.8	153.6	154.8	150.0
27.	115.2	102.8	96.0	105.2	92.4	128.4	144.0	126.4	132.8
				Serum Very Low Density lipoprotein (mg/dl)					
26.	35.2	36.0	37.2	37.2	38.0	37.2	36.4	39.2	36.0
27.	42.0	41.8	40.0	40.8	39.2	41.6	40.0	35.6	37.2

F = Fasting values,
A = 15 minutes postprandial values,
B = 30 minutes postprandial values,
C = 45 minutes postprandial values,

I = 1 hour postprandial values,
II = 2 hour postprandial values,
III = 3 hour postprandial values,
IV = 4 hour postprandial values,
V = 5 hour postprandial values,